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(54) The gal operon of streptomyces.

(57) A recombinant DNA molecule comprising the *Streptomyces gal* operon *gal*K gene; *gal*E gene; *gal*T gene; P1 promoter; P2 promoter; P2 promoter expression unit; P1 promoter regulated region; or the entire *Streptomyces gal* operon is prepared.

TITLE

THE GAL OPERON OF STREPTOMYCES

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of Serial Number 834,706, filed February 28, 1986, which is pending.

BACKGROUND OF THE INVENTION

This invention relates to a recombinant DNA molecule comprising the *Streptomyces gal* operon.

Hodgson, *J. Gen. Micro.*, *128*, 2417-2430 (1982), report that *Streptomyces coelicolor* A3(2) has a glucose repression system which allows repression at the level of transcription of the arabinose uptake system, one of the glycerol uptake systems, and also repression of the galactose uptake system in wild type strains. There is no report in Hodgson of actual galactose metabolism by *S. coelicolor* A3(2).

Okeda et al. *Mol. Gen. Genet.*, *196*, 501-507 (1984), report that glucose kinase activity, 2-deoxyglucose-sensitivity, glucose utilization and glucose repression were all restored to *S. coelicolor* A3(2) *glk* (glucose kinase) mutants transformed by a 3.5 kb DNA fragment which contained the *glk* gene cloned from *S. coelicolor* into a phage vector.

Seno et al., Mol. Gen. Genet., 193, 119-128 (1984), report the glycerol (gyl) operon of Streptomyces coelicolor, and state that such operon is substrate-inducible and catabolite-repressible.

Debouck et al., Nuc. Acids. Res., 13(6), 1841-1853 (1985), report that the gal operon of E. coli consists of three structurally contiguous genes which specify the enzymes required for the metabolism of galactose, i.e., galE (uridine diphosphogalactose-4-epimerase), galT (galactose-1-phosphate uridyltransferase) and galK (galactokinase); that such genes are expressed from a polycistronic mRNA in the order E, T, K; that the expression of the promoter distal gene of the operon, galK, is known to be coupled translationally to the galT gene immediately preceding it; that such translational coupling results from a structural overlap between the end of the galT coding sequence and the ribosome binding region of galK; and that the translational coupling of galT and galK ensures the coordinate expression of these genes during the metabolism of galactose.

SUMMARY OF THE INVENTION

This invention relates to a recombinant DNA molecule comprising a Streptomyces gal operon galK gene; galE gene; galT gene; P2 promoter expression unit, or P2 promoter or any functional derivative thereof as well as a recombinant DNA molecule comprising a Streptomyces gal operon P1 promoter, P1 promoter regulated region or the entire gal operon or any regulatable and functional derivative thereof.

This invention also relates to a recombinant DNA molecule comprising the Streptomyces gal operon or any regulatable and functional derivative thereof and a functional DNA molecule operatively linked to such operon; a recombinant DNA vector comprising and such DNA molecule, and, optionally, additionally comprising a replicon; a method of preparing a host cell transformed with such vector; the transformed host prepared by such method; a method of expressing such functional DNA sequence which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed; and to a method of regulating the expression of such functional DNA sequence which comprises cultivating such transformed host under conditions which regulate such expression.

This invention also relates to a recombinant DNA molecule comprising the _Streptomyces_ _gal_ operon P2 promoter expression unit or any functional derivative thereof and a functional DNA molecule operatively linked to such unit; a recombinant DNA vector comprising such DNA molecule, and, optionally, additionally comprising a replicon; a method of preparing a host cell transformed with such vector; the transformed host prepared by such method; and to a method of expressing such functional DNA sequence which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed.

This invention also relates to a recombinant DNA molecule comprising the _Streptomyces_ _gal_ operon P1 promoter regulated region or any regulatable and functional derivative thereof and a functional DNA molecule operatively linked to such region; a recombinant DNA vector comprising such DNA molecule, and, optionally, additionally comprising a replicon; a method of preparing a host cell transformed with such vector; the transformed host prepared by such method; a method of expressing such functional DNA sequence which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed; and to a method of regulating the expression of such functional DNA sequence which comprises cultivating such transformed host under conditions which regulate such expression.

This invention also relates to a recombinant DNA molecule comprising the Streptomyces gal operon P1 promoter or any regulatable and functional derivative thereof and a foreign functional DNA molecule operatively linked to such region; a recombinant DNA vector comprising such DNA molecule, and, optionally, additionally comprising a replicon; a method of preparing a host cell transformed with such vector; the transformed host prepared by such method; a method of expressing such functional DNA sequence which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed; and to a method of regulating the expression of such functional DNA sequence which comprises cultivating such transformed host under conditions which regulate such expression.

This invention also relates to a recombinant DNA molecule comprising the Streptomyces gal operon P2 promoter or any functional derivative thereof and a foreign functional DNA molecule operatively linked to such region; a recombinant DNA vector comprising such DNA molecule, and, optionally, additionally comprising a replicon; a method of preparing a host cell transformed with such vector; the transformed host prepared by such method; and to a method of expressing such functional DNA sequence which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed.

This invention also relates to a method of enabling a non-galactose utilizing host microorganism or cell to utilize galactose which comprises transforming such host with a recombinant DNA molecule comprising a Streptomyces gal operon or any portion of the Streptomyces gal operon, or any functional derivative thereof, which is adequate to enable such transformed host to utilize galactose, This invention also relates to the recombinant DNA vector employed in such method and to the host prepared by such method.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents a restriction endonuclease map of the *Streptomyces lividans* 1326 galactose (*gal*) operon and indicates approximate locations for structural genes and promoters within the operon.

Figure 2 represents a restriction endonuclease map of plasmid pK21.

Figure 3 represents a comparison of the restriction endonuclease maps of the *S. lividans gal* operon and a restriction fragment containing the *S. coelicolor galK* gene.

DETAILED DESCRIPTION OF THE INVENTION

It has now been discovered that the *Streptomyces* genome contains a operon for the metabolism of galactose (i.e., a *gal* operon) which comprises three structural genes (*gal*T, *gal*E and *gal*K) and two promoters (P1 and P2). The *gal*T gene product is known as galactose-1-phosphate uridyltransferase (transferase), the *gal*E gene product is known as uridine diphosphogalactose-4-epimerase (epimerase), and the *gal*K gene product is known as galactose-1-kinase (galactokinase). The function of the gene products of *gal*T, *gal*E and *gal*K in galactose metabolism in *Streptomyces* is explained by the following diagram:

1. galactose + ATP $\xrightarrow{\text{galactokinase}}$ galactose-1-phosphate + ADP
2. galactose-1-phosphate + UDP-glucose $\xrightarrow{\text{transferase}}$ UDP-galactose + glucose-1-phosphate
3. UDP-galactose $\xrightarrow{\text{epimerase}}$ UDP-glucose

By the term "promoter" is meant any region upstream of a structural gene which permits binding of RNA polymerase and transcription to occur.

By the term "structural gene" is meant a coding sequence for a polypeptide which serves to be the template for the synthesis of mRNA.

By the term "operon" is meant a group of closely linked genes responsible for the synthesis of one or a group of enzymes which are functionally related as members of one enzyme system. An operon comprises an operator gene, a number of structural genes (equivalent to the number of enzymes in the system) and a regulator gene. By "operator" or "operator gene" is meant a DNA sequence which controls the biosynthesis of the contiguous structural gene(s) within an operon. By "regulator gene" is meant a gene which controls the operator gene in an operon through the production of a repressor which can be either active (enzyme induction) or inactive (enzyme repression). The transcription of the structural gene(s) in an operon is switched on or off by the operator gene which is itself controlled in one or more of three ways: 1) in inducible enzyme systems, the operator is switched off by a repressor produced by the regulator gene and which can be inactivated by some metabolite or signal substance (an inducer) coming from elsewhere in the cell or outside the cell, so that the presence of the inducer results in the operon becoming active; or 2) in repressed enzyme systems, the operator is switched off by a repressor-corepressor complex which is a combination of an inactive repressor produced by the regulator gene with a corepressor from elsewhere, so that the presence of the corepressor renders the operon inactive; or 3) in activated gene systems, the promoter is switched on by an activator produced by a regulator gene which can be activated by some metabolic or signal substance.

The _Streptomyces_ _gal_ operon is naturally present in the _Streptomyces_ genome.

By the term "_Streptomyces_ _gal_ operon" is meant that region of the _Streptomyces_ genome which comprises the

P1 promoter, P2 promoter, galT, galE and galK structural genes and any other regulatory regions required for transcription and translation of such structural genes.

By the term "regulatory region" is meant a DNA sequence, such as a promoter or operator, which regulates transcription of a structural gene.

The following model is suggested for gene expression within the Streptomyces gal operon. The P1 promoter is a galactose inducible promoter (i.e., it is induced in the presence of galactose and repressed in the presence of glucose). According to S1 data, the P2 promoter is constitutive, i.e., it is "turned on" regardless of the presence or absence of galactose or any other carbon source.

A cosmid library was constructed for Streptomyces lividans 1326 DNA by using cosmid pJW357 (which encodes the ability to replicate in both Streptomyces and E. coli). This library was then transfected into E. coli K21 which is a derivative of the E. coli strain MM294 which contained a bacteriophage P1 transduced galactokinase (galK) mutation. Transfected cells were plated under media conditions which select for both the presence of the cosmid and the presence of an active galK gene. Weakly positive colonies were isolated and the cosmid DNA derived from these colonies was transformed into the K21 strain. These transformations yielded two cosmids which consistently produced positive growth with galactose as the only carbon source. These galK$^+$ cosmids were then transformed into a Streptomyces host (i.e., Streptomyces lividans 1326-12K) which had been isolated by the inventors of the subject invention as unable to grow on medium in which galactose was the only carbon source by using 2-deoxy-galactose selection [see, Brawner et al., Gene, 40 191 (1985), in press]. Under conditions which differentiate strains able and unable to produce galactokinase, only one of the cosmids caused the Streptomyces lividans 1326-12K host to become galK^{+}. Further studies have demonstrated that this cosmid encodes a gene with galactokinase activity. Additional studies, including DNA sequence analysis and protein studies demonstrate that this Streptomyces gene shares homology with the E. coli and yeast galactokinase genes. Regulation studies indicate that the cosmid encoded galactokinase gene regulated in the same manner as the chromosome encoded gene.

A. S. lividans gal operon was originally isolated from a ca. 9 kilobase (Kb) region of Streptomyces lividans 1326. The ca. 9 Kb region of Streptomyces lividans 1326 containing the Streptomyces gal operon has been mapped substantially as follows in Table A. By "substantially" is meant (i) that the relative positions of the restriction sites are approximate, (ii) that one or more restriction sites can be lost or gained by mutations not otherwise significantly affecting the operon, and (iii) that additional sites for the indicated enzymes and, especially for enzymes not tested, may exist. The restriction enzymes used herein are commercially available. All are described by Roberts, Nuc. Acids. Res., 10(5): p117 (1982).

TABLE A

Map Position	Restriction Enzyme	Location (kb)
1	*Hind*III	-.40
1a	*Nru*I	0
2	*Bgl*II	.75
3	*Eco*RI	1.05
4	*Pvu*II	1.15
5	*Mlu*I	2.30
6	*Pvu*II	2.80
7	*Eco*RI	4.00
8	*Pvu*II	4.10
8a	*Sac*I	4.25
9	*Pvu*II	5.00
10	*Xho*I	5.50
11	*Bam*HI	5.80
12	*Bam*HI	6.50
13	*Mlu*I	6.90
13a	*Pvu*II	7.20
14	*Mlu*I	7.80
15	*Bam*HI	8.00
16	*Sph*I	8.30

Figure 1 represents a restriction endonuclease map of the *Streptomyces lividans* 1326 *gal* operon and indicates locations for structural genes (*gal*T, *gal*E and *gal*K) and promoters (P1 and P2) comprised within the operon.

Referring to Table A and Figure 1, the location of the promoters and structural genes of the Streptomyces lividans 1326 gal operon are mapped substantially as follows in Table B:

TABLE B

	Location (Kb)
P1 transcription start site	.10
galT translation initiation codon	.15
P2 transcription start site	1.25
galE translation initiation codon	1.50
galK translation initiation codon	2.40
3' end of galK message	3.60

Microorganisms of the genus Streptomyces have historically been used as a source of antibiotics for the pharmaceutical industry. Consequently, the technical skills necessary to scale-up the production of biological products using Streptomyces as the vehicle for the production of such products are presently available. However, before Streptomyces can be used as a vehicle for the production of bioactive molecules using the new recombinant DNA technologies, there is a need to define regulatory elements in Streptomyces analogous to those which have proved useful in E. coli. These regulatory elements include ribosomal binding sites and regulated transcriptional elements.

The existence of a galE, galT or galK gene or gene product or gal operon in Streptomyces has not been previously reported. The instant invention, i.e., the cloning of the Streptomyces gal operon, enables construction of regulatable expression/cloning vectors in Streptomyces, other actinomycetes, and other host organisms. Furthermore, the instant invention led to the discovery that the Streptomyces gal operon is polycistronic. Perhaps the most important feature of the cloning of the Streptomyces gal operon is the observation that there are sequences essential for regulation of the Streptomyces galK gene. Direct analogy to the initial use of the lac promoter from E. coli as an expression system can be made. In fact, Brosius et al., Proc. Natl. Acad. Sci. USA, 81, 6929-6933 (1984), utilized the regulatory elements of the E. coli lac promoter to regulate the exceptionally strong E. coli ribosomal promoters. Because it is likely that the Streptomyces gal operon ribosomal promoters are also exceptionally strong, such promoters enable the construction of regulatable expression vectors which will be very useful in Streptomyces, other actinomycetes, and other host organisms. The instant invention also enabled the unexpected discovery that the 2-deoxygalactose selection which has been used in E. coli to select for galK mutants also operates in Streptomyces to select for galK mutants [see, Brawner et al., Gene 40, 191 (1985), in press]. This observation, combined with the ability to clone the Streptomyces galK gene and the promoter and regulatory regions required for its transcription and translation on a cosmid, as described herein, allows the direct insertion of any structural gene into the chromosomally located galK gene of Streptomyces by homologous recombination. This manipulation will allow molecular biologists to stably insert DNA fragments of interest into the Streptomyces chromosome. Such an approach will allow researchers to tag or mark a Streptomyces strain of interest or to insert expression cassettes into the organism without the need of maintaining an antibiotic selection such as that presently required by most Streptomyces expression vectors.

This invention relates to a recombinant DNA molecule comprising the Streptomyces gal operon or any regulatable and functional derivative thereof.

By "regulatable and functional derivative" is meant any derivative of the Streptomyces gal operon which functions in substantially the same way as the naturally occurring Streptomyces gal operon in terms of regulatable production of the galT, galE and galK gene products. Such derivatives include partial sequences of the gal operon, as well as derivatives produced by modification of the gal operon coding sequence. Techniques for modifying the gal operon which are known in the art include, for example, treatment with chemical mutagens, irradiation or direct genetic engineering, such as by inserting, deleting or substituting nucleic acids by the use of enzymes or recombination techniques. The naturally occurring Streptomyces gal operon can be isolated from any galactose utilizing Streptomyces strain by employing the techniques described herein. Numerous strains of various Streptomyces species are publicly available from many sources. For example, the American Type Culture Collection, Rockville, Maryland, U.S.A. has approximately 400 different species of Streptomyces available to the public. The ability of a particular strain of Streptomyces to utilize galactose can be readily determined by conventional techniques, such as by growing such strain on a medium containing galactose as the sole carbon source. The preferred Streptomyces species from which to isolate a gal operon include S. lividans, S. coelicolor, S. azuraeus and S. albus, S. carzinostaticus, S. antifibrinolyticus and S. longisporus. S. lividans is most preferred. The Streptomyces gal operon, and smaller portions thereof, is useful as a nucleic acid probe to obtain homologous sequences from other cells and organisms. The Streptomyces gal operon is also useful as a selection marker in an appropriate host mutant, and for providing regulatory elements. By "appropriate host mutant" is meant a host which does not utilize galactose because it (a) does not contain a gal operon or (b) contains a nonfunctional gal operon, or (c) contains a defect within a homologous structural gene or regulatory region comprised by the Streptomyces gal operon such as a defective P1 promoter, P2 promoter, galT gene, galK gene and/or galE gene. Thus, a recombinant DNA molecule (comprising the Streptomyces gal operon and a foreign functional DNA sequence operatively linked thereto), which can be prepared by conventional techniques, can be transformed into an appropriate host mutant by conventional techniques for incorporation into the host genome by homologous recombination to enable regulatable expression of the foreign functional DNA sequence without the need of maintaining an expensive antibiotic selection. Such operon may therefore also be incorporated on recombinant DNA expression vectors for regulatable expression of a foreign functional DNA sequence operatively linked to such operon in an appropriate host mutant transformed with such vector without the need of maintaining an expensive antibiotic selection. Such operon is also useful for transforming those cells, viruses and microorganisms, such as strains of Streptomyces, other actinomycetes, and other prokaryotic organisms, such as gal^- E. coli strains, which do not utilize galactose into galactose utilizing strains. Such transformation may have pleiotrophic effects on the transformed host. By the term "functional DNA sequence" is meant any discrete region of DNA derived directly or indirectly from Streptomyces or any other source which functions in a host organism transformed therewith as a gene expression unit, structural gene, promoter or a regulatory region. Preferred functional DNA sequences include those coding for polypeptides of pharmaceutical importance, such as, but not limited to, insulin, growth hormone, tissue plasminogen activator, alpha -1-anti-trypsin or antigens used in vaccine production. By the term "foreign functional DNA sequence" is meant a functional DNA sequence not derived from the Streptomyces gal operon coding region.

This invention also relates to a recombinant DNA molecule comprising the Streptomyces gal operon P2 promoter expression unit or any functional derivative thereof. By the term "P2 promoter expression unit" is meant that region of the Streptomyces gal operon comprising the Streptomyces gal operon P2 promoter, galE and galK structural genes and any other regulatory regions required for transcription and translation of such structural genes. By "functional derivative" is meant any derivative of the Streptomyces gal operon P2 promoter expression unit which functions in substantially the same way as the naturally occurring region in terms of production of the Streptomyces gal operon galE and galK gene products. Such derivatives include partial sequences of the Streptomyces gal operon P2 promoter expression unit, as well as derivatives produced by modification of the Streptomyces gal operon P2 promoter expression unit coding sequence. Techniques for effecting such modification are known in the art, and some have been outlined above. The naturally occurring Streptomyces gal operon P2 promoter expression unit can be isolated from the naturally occurring Streptomyces gal operon by conventional techniques. The Streptomyces gal operon P2 expression unit is useful as a selection marker in an appropriate host mutant and for providing regulatory elements. By "appropriate host mutant" is meant a host which does not utilize galactose because it contains a defect within a homologous structural gene or regulatory region comprised by the Streptomyces P2 promoter expression unit such as a defective P2 promoter, galE gene and/or galK gene. Thus, a recombinant DNA molecule (comprising the Streptomyces gal operon P2 promoter expression unit and a foreign functional DNA sequence .

operatively linked thereto), which can be prepared by conventional techniques, can be transformed into an appropriate host mutant by conventional techniques for incorporation into the host genome by homologous recombination to enable constitutive expression of the foreign functional DNA sequence without the need of maintaining an expensive antibiotic selection. Such expression unit may also be incorporated on recombinant DNA expression vectors for constitutive expression of foreign functional DNA sequences. The Stretomyces gal operon P2 promoter expression unit is also useful for complementation of an appropriate host mutant which can then be used for constitutive expression of a foreign functional DNA sequence operatively linked to such expression unit in an appropriate host mutant transformed with such vector without the need of maintaining an expensive antibiotic selection.

This invention also relates to a recombinant DNA molecule comprising the Streptomyces gal operon P1 promoter regulated region or any regulatable and functional derivative thereof. By the term "P1 promoter regulated region" is meant that region of the Streptomyces gal operon comprising the Streptomyces gal operon P1 promoter, galT, galE and galK structural genes and any other regulatory regions required for transcription and translation of such structural genes. By "regulatable and functional derivative" is meant any derivative of the Streptomyces gal operon P1 promoter regulated region which functions in substantially the same way as the naturally occurring region in terms of regulatable production of the Streptomyces gal operon galT, galE and galK gene products. Such derivatives include partial sequences of the Streptomyces gal operon P1 promoter regulated region, as well as derivatives produced by modification of the Streptomyces gal operon P1 promoter regulated region coding sequence. Techniques for effecting such modification are known in the art, and some have been outlined above. The naturally occurring Streptomyces gal operon P1 promoter regulated region can be isolated from the naturally occurring Streptomyces gal operon by conventional techniques, such as by excising the P2 promoter from the naturally occurring Streptomyces gal operon or inactivating the P2 promoter by a point mutation or by inserting a foreign DNA sequence within the promoter. The Streptomyces gal operon P1 promoter regulated region is useful for the utilities outlined above for the Streptomyces gal operon.

This invention also relates to a recombinant DNA molecule comprising the Streptomyces gal operon P2 promoter or any functional derivative thereof. By "functional derivative" is meant any derivative of the Streptomyces gal operon P2 promoter which functions in substantially the same way as the naturally occurring P2 promoter in terms of enabling the binding of RNA polymerase thereto and transcription of a functional DNA sequence operatively linked to such promoter. Such derivatives include partial sequences of the Streptomyces gal operon P2 promoter, as well as derivatives produced by modification of the gal operon P2 promoter coding sequence. Techniques for effecting such modification are known in the art, and some have been outlined above. The naturally occurring Streptomyces gal operon P2 promoter can be isolated from the naturally occurring Streptomyces gal operon by conventional techniques. A recombinant DNA molecule (comprising the Streptomyces gal operon P2 promoter and a foreign functional DNA sequence operatively linked thereto), which can be prepared by conventional techniques, can be transformed into an appropriate host mutant by conventional techniques for incorporation into the host genome by homologous recombination to enable constitutive expression of the foreign functional DNA sequence. The Streptomyces gal operon P2 promoter is also useful for incorporation into recombinant DNA expression vectors for constitutive expression of a foreign functional DNA sequence operatively linked thereto in viruses and eukaryotic or prokaryotic cells or organisms, especially in *Streptomyces* or other actinomycetes, transformed with such vector.

This invention also relates to a recombinant DNA molecule comprising the *Streptomyces* *gal* operon P1 promoter or any regulatable and functional derivative thereof. By "regulatable and functional derivative" is meant any derivative of the *Streptomyces* *gal* operon P1 promoter which functions in substantially the same way as the naturally occurring P1 promoter in terms of enabling the binding of RNA polymerase thereto and regulating the transcription of a functional DNA sequence operatively linked to such promoter. Such derivatives include partial sequences of the *Streptomyces* *gal* operon P1 promoter, as well as derivatives produced by modification of the *gal* operon P1 promoter coding sequence. Techniques for effecting such modification are known in the art, and some have been outlined above. The naturally occurring *Streptomyces* *gal* operon P1 promoter can be isolated from the naturally occurring *Streptomyces* *gal* operon by conventional techniques. A recombinant DNA molecule (comprising the *Streptomyces* *gal* operon P1 promoter and a foreign functional DNA sequence operatively linked thereto), which can be prepared by conventional techniques, can be transformed into an appropriate host mutant by conventional techniques for incorporation into the host genome by homologous recombination to enable regulatable expression of the foreign functional DNA sequence. The *Streptomyces* *gal* operon P1 promoter is also useful for incorporation into recombinant DNA expression vectors for regulatable expression of a foreign functional DNA sequence operatively linked thereto in viruses and eukaryotic or prokaryotic cells or organisms, especially

Streptomyces or other actinomycetes, transformed with such vector.

This invention also relates to a recombinant DNA molecule comprising the Streptomyces gal operon galE, galT or galK gene, or any functional derivative thereof. By "functional derivative" is meant any derivative of the Streptomyces gal operon galE, galT or galK gene which functions in substantially the same way as the naturally occurring gene in terms of production of an active galE, galT, or galK type gene product. Such derivatives include partial sequences of the Streptomyces gal operon galE, galT, or galK gene, as well as derivatives produced by modification of the gal operon sequence. Techniques for effecting such modification are known in the art, and some have been outlined above. The naturally occurring Streptomyces gal operon galE, galT and/or galK gene can be isolated from the naturally occurring Streptomyces gal operon by conventional techniques. The Streptomyces gal operon galE, galT and/or galK gene can be used as a selection marker in an appropriate host mutant. By "appropriate host mutant is meant a host which does not utilize galactose because it contains a defect within a homologous galE, galT and/or galK gene. Thus, a recombinant DNA molecule (comprising the Streptomyces gal operon galE, galT and/or galK gene and a foreign functional DNA sequence, both of which are operatively linked to appropriate regulatory region), which can be prepared by conventional techniques, can be transformed into an appropriate host mutant by conventional techniques for incorporation into the host genome by homologous recombination to enable detection of transformants without the need of maintaining an expensive antibiotic selection. Likewise, a recombinant DNA vector comprising the Streptomyces gal operon galE, galT and/or galK gene and a foreign functional DNA sequence, both of which are operatively linked to appropriate regulatory regions, as well as a replicon, can be transformed into an appropriate host mutant by conventional techniques to enable detection of transformants without the need of maintaining an expensive antibiotic selection. The Streptomyces gal operon galE, galK and/or galT gene is also useful for complementation of an appropriate host mutant.

The Streptomyces gal operon galE gene is also useful for providing a ribosome binding site and initiation codon which can be fused to a foreign functional DNA sequence to enable the expression of such coding sequence when incorporated into an appropriate expression vector and transformed into an appropriate host. If such foreign functional DNA sequence is fused to the galE gene ribosome binding site and initiation codon in a recombinant DNA expression vector comprising the Streptomyces gal operon P2 promoter expression unit, or the entire gal operon, such DNA sequence will be constitutively expressed when such vector is transformed into an appropriate host organism. If such DNA sequence is fused to the galE gene ribosome binding site and initiation codon in a recombinant DNA expression vector comprising the Streptomyces gal operon P2 promoter regulated region, expression of such DNA sequence can be regulated when such vector is transformed into an appropriate host organism by controlling the presence or absence of galactose or glucose.

The Streptomyces gal operon galT gene is also useful for providing a ribosome binding site and initiation codon which can be fused to a foreign functional DNA sequence to enable the expression of such coding sequence when incorporated onto an appropriate expression vector and transformed into an appropriate host. If such DNA sequence is fused to the galT gene ribosome binding site and initiation codon in a recombinant DNA expression vector comprising the

Streptomyces gal operon P1 promoter regulated region, or the entire gal operon, expression of such coding sequence can be regulated in a host transformed with such vector as outlined above.

This invention also relates to a recombinant DNA vector comprising a replicon, Streptomyces gal operon, or a functional and regulatable derivative thereof, and a foreign functional DNA sequence operatively linked to such operon. Such vector can be prepared by conventional techniques. The replicon employed should be one known for its ability to stably and extrachromosomally, maintain a vector in the host organism which is to be the host transformed with the vector.

This invention also relates to a transformed host microorganism comprising a recombinant DNA vector wherein said vector contains a replicon, the Streptomyces gal operon, or a functional and regulatable derivative thereof, and a foreign functional DNA sequence operatively linked to such operon; and to the method of preparing such host which comprises transforming an appropriate host microorganism with such vector. Appropriate host microorganisms which may be employed in the method of this invention include viruses, and eukaryotic and prokaryotic cells or organisms, especially actinomycetes, such as those of the genus Streptomyces. The most preferred host microorganisms belong to the genus Streptomyces. Preferred species of Streptomyces include Streptomyces lividans, S. coelicolor, S. azuraeus and S. albus. Transformation of such host microorganism with such vector can be accomplished using conventional techniques such as the method of Chater et al., Curr. Top. Micro. Imm., 96, 69-95 (1982). This invention also related to a method of expressing the functional DNA sequence contained by such transformed host of this invention which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed. By "suitable conditions" is meant those conditions which will allow the host to grow and which enable the expression of the functional DNA sequence. Such suitable conditions can be determined by one of skill in the art using conventional techniques and will depend on various factors, such as the host organism employed and the functional DNA sequence to be expressed. This invention is also related to a method of regulating the expression of the functional DNA sequence contained by such transformed host which comprises cultivating a transformed host containing such functional DNA sequence under appropriate conditions such that its expression is regulatable. By "appropriate conditions" is meant those conditions which enable the Streptomyces gal operon (and thus the foreign functional DNA sequence) to be regulatable. By "regulatable" is meant responsive to the presence of galactose or its metabolites and the presence of glucose or its metabolites in the growth media of the transformed host cell. Such regulation can be carried out by addition or deletion of galactose or glucose to the transformed host's culture medium. The optimal levels of galactose and/or glucose for up or down-regulation of the expression of the foreign functional DNA coding sequence by the transformed host of this invention can be readily determined by one of skill in the art using conventional techniques.

This invention also relates to a recombinant DNA vector comprising a replicon, a Streptomyces gal operon P2 promoter expression unit, or a functional derivative thereof, and a foreign functional DNA sequence operatively linked to such unit. Such a vector can be prepared by conventional techniques. The replicon employed should be one known for its ability to stably, and extra-chromosomally, maintain a vector in the host organism which is to be transformed with the vector.

This invention also relates to a transformed host microorganism comprising a recombinant DNA vector wherein said vector contains a replicon, the Streptomyces gal operon P2 promoter expression unit, or a functional derivative thereof, and a foreign functional DNA sequence operatively linked to such unit; and to the method of preparing such host which comprises transforming an appropriate host microorganism with such vector. By the term "operatively linked" is meant that a functional DNA sequence is transcriptionally or translationally linked to an expression control sequence (i.e., the Streptomyces gal operon, P2 promoter expression unit, P1 promoter regulated region, P1 promoter or P2 promoter) in such a way so that the expression of the functional DNA sequence is under control of the expression control sequence. Thus, for example, a foreign functional DNA sequence can be transcriptionally or translationally linked to the Streptomyces gal operon by inserting such operon within the Streptomyces gal operon P1 or P2 promoter transcript. By the term "replicon" is meant that region of DNA on a plasmid which functions to maintain, extrachromosomally, such plasmid in a host microorganism or cell transformed therewith. It has also been discovered that the Streptomyces gal operon, and smaller portions thereof, is useful as a nucleic acid probe to obtain homologous sequences from other cells and organisms. Appropriate host microorganisms which may be employed in the method of this invention include any virus or eukaryotic or prokaryotic cell or organism, especially any actinomycetes such as those of the genus Streptomyces. The most preferred host microorganisms belong to the genus Streptomyces. Preferred species of Streptomyces include Streptomyces lividans, S. coelicolor, S. azuraeus and S. albus. Transformation of such host microorganism with such vector can be accomplished using conventional techniques such as the method of Chater et al., Curr. Top. Micro. Imm., 96, 69-95 (1982). This invention also related to a method of expressing the functional DNA sequence contained by such transformed host of this invention which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed. By "suitable conditions" is meant those conditions which will allow the host to grow and which enable the expression of the functional DNA sequence. Such suitable conditions can be determined by one of skill in the art using conventional techniques and will depend on various factors, such as the host organism employed and the functional DNA sequence to be expressed.

This invention also relates to a recombinant DNA vector comprising a replicon, a *Streptomyces* *gal* operon P1 promoter regulated region, or a functional and regulatable derivative thereof, and a foreign functional DNA sequence operatively linked to such region. Such a vector can be prepared by conventional techniques. The replicon employed should be one known for its ability to stably and extrachromosomally maintain a vector in the host organism which is to be the host transformed with the vector.

This invention also relates to a transformed host microorganism comprising a recombinant DNA vector wherein said vector contains a replicon, a *Streptomyces* *gal* operon P1 promoter regulated region, or a functional and regulatable derivative thereof, and a foreign functional DNA sequence operatively linked to such region; and to the method of preparing such host which comprises transforming an appropriate host microorganism with such vector. Appropriate host microorganisms which may be employed include any virus or eukaryotic or prokaryotic cell or organism especially actinomycetes such as those of the genus *Streptomyces*. The most preferred host microorganisms belong to the genus *Streptomyces*.

Preferred species of <u>Streptomyces</u> include <u>Streptomyces lividans</u>, <u>S. coelicolor</u>, <u>S. azuraeus</u> and <u>S. albus</u>. Transformation of such host microorganism with such vector can be accomplished using conventional techniques such as the method of Chater et al., <u>Curr. Top. Micro. Imm.</u>, <u>96</u>, 69-95 (1982). This invention also related to a method of expressing the foreign functional DNA sequence contained by such transformed host of this invention which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed. By "suitable conditions" is meant those conditions which will allow the host to grow and which enable the expression of the functional DNA sequence. Such suitable conditions can be determined by one of skill in the art using conventional techniques and will depend on various factors, such as the host organism employed and the functional DNA sequence to be expressed. This invention also related to a method of regulating the expression of the functional DNA sequence contained by such transformed host which comprises cultivating a transformed host containing such functional DNA sequence under appropriate conditions such that its expression is regulatable. By "appropriate conditions" is meant those conditions which enable the <u>Streptomyces</u> <u>gal</u> operon P1 promoter regulated region (and thus the foreign functional DNA sequence) to be regulatable. By "regulatable" is meant responsive to the presence or absence of galactose or its metabolites and the presence or absence of glucose or its metabolites in the growth media of the transformed host cell. Such regulation can be carried out by addition or deletion of galactose or glucose to the transformed host's culture medium.

This invention also relates to a recombinant DNA vector comprising a replicon, a <u>Streptomyces</u> <u>gal</u> operon P2 promoter, or a functional derivative thereof, and a foreign functional DNA sequence operatively linked to such promoter. Such a vector can be prepared by conventional techniques. The replicon employed should be one known for its ability to stably and extrachromosomally maintain a vector in the host organism which is to be the host transformed with the vector.

This invention also relates to a transformed host microorganism comprising a recombinant DNA vector wherein said vector contains a replicon, a Streptomyces gal operon P2 promoter, or a functional derivative thereof, and a foreign functional DNA sequence operatively linked to such region; and to the method of preparing such host which comprises transforming an appropriate host microorganism with such vector. Appropriate host microorganisms which may be employed include actinomycetes such as those of the genus Streptomyces. The most preferred host microorganisms belong to the genus Streptomyces. Preferred species of Streptomyces include Streptomyces lividans, S. coelicolor, S. azuraeus and S. albus. Transformation of such host microorganism with such vector can be accomplished using conventional techniques such as the method of Chater et al., Curr. Top. Micro. Imm., 96, 69-95 (1982). This invention also related to a method of expressing the foreign functional DNA sequence contained by such transformed host of this invention which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed. By "suitable conditions" is meant those conditions which will allow the host to grow and which enable the expression of the functional DNA sequence. Such suitable conditions can be determined by one of skill in the art using conventional techniques and will depend on various factors, such as the host organism employed and the functional DNA sequence to be expressed.

This invention also relates to a recombinant DNA vector comprising a replicon, Streptomyces gal operon P1 promoter, or any regulatable and functional derivative thereof, and a foreign functional DNA sequence operatively linked to such region. Such a vector can be prepared by conventional techniques. The replicon employed should be one known for its ability to stably and extrachromosomally maintain a vector in the host organism which is to be the host transformed with the vector.

This invention also relates to a transformed host microorganism comprising a recombinant DNA vector wherein said vector contains a replicon, the Streptomyces gal operon P1 promoter, or any regulatable and functional derivative thereof, and a foreign functional DNA sequence operatively linked to such region; and to the method of preparing such host which comprises transforming an appropriate host microorganism with such vector. Appropriate host microorganisms which may be employed include viruses or prokaryotic or eukaryotic cells or organisms, especially actinomycetes such as those of the genus Streptomyces. The most preferred host microorganisms belong to the genus Streptomyces. Preferred species of Streptomyces include Streptomyces lividans, S. coelicolor, S. azuraeus and S. albus. Transformation of such host microorganism with such vector can be accomplished using conventional techniques such as the method of Chater et al., Curr. Top. Micro. Imm., 96, 69-95 (1982). This invention also relates to a method of expressing the foreign functional DNA sequence contained by such transformed host of this invention which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed. By "suitable conditions" is meant those conditions which will allow the host to grow and which enable the expression of the functional DNA sequence. Such suitable conditions can be determined by one of skill in the art using conventional techniques and will depend on various factors, such as the host organism employed and the foreign functional DNA sequence to be expressed. This invention also relates to a method of regulating the expression of the functional DNA sequence contained by such transformed host which comprises cultivating a transformed host containing such foreign functional DNA sequence under appropriate conditions such that its expression is regulatable. By "appropriate conditions" is meant those conditions which enable the gal operon P1 promoter (and thus the functional DNA sequence) to be regulatable. By "regulatable" is meant responsive to the presence or absence of galactose or its metabolites and the presence of glucose or its metabolites in the growth media of the transformed host cell. Such regulation can be carried out by addition or deletion of galactose or glucose to the transformed host's culture medium.

EXAMPLES

In the following Examples, specific embodiments of the invention are more fully disclosed. These Examples are intended to be illustrative of the subject invention and should not be construed as limiting its scope. In all Examples, temperature is in degrees Centigrade (°C).

By utilizing conventional methods, such as those outlined in the following Examples, one of skill in the art can isolate the gal operon from any galactose utilizing strain of Streptomyces. Furthermore, by utilizing techniques similar to those employed herein to isolate the Streptomyces gal operon, one of skill in the art can attempt to use the Streptomyces gal operon to isolate a gal operon from other galactose utilizing other strains of Streptomyces, especially S. coelicolor, S. azuraeus, S. albus and other S. lividans strains.

Molecular genetic manipulations and other techniques employed in the following Examples are described in Hopwood et al., Genetic Manipulation of Streptomyces: A Laboratory Manual, John Innes Foundation, Norwich, England (1985).

ABBREVIATIONS

In the following Examples, the following abbreviations may be employed:

LB: 10 grams (g) tryptone, 5 g yeast extract, 5g NaCl

MBSM (modified MBSM): See, Brawner et al., <u>Gene</u>, <u>40</u>, 191 (1985) (in press)

MOPS: (3)-N-morpholino-(proprane-sulfonic acid)

YEME + $MgCl_2$ + Glycine: [per liter(l)] 3 g yeast extract, 5 g peptone, 3 g malt extract, 10 g glucose, 10 g $MgCl_2$" $62H_2O$, 340 g sucrose.

SL: Mix together $(NH_4)_2SO_4$(1g/l); L-asparagine (2 g/l); K_2HPO_4 (9 g/l); NaH_2PO_4 (1 g/l) for 0.2% agar and autoclave. Then mix with yeast extract (20 g/l), $MgCl_2$ (5 g/l); $CuCl_2$ (0.1 g/l); Trace elements [20 ml/l - include $ZnCl_2$-40 mg/l; $FeCl_3$"$6H_2O$ (200 mg/l); $CuCl_2$"$2H_2O$ (10 mg/l); NaB_4O_7"$10H_2O$ (10 mg/l); $(NH_4)_6MO_7O_{24}$" 4 H_2O(10 mg/l)] filter and sterilize.

YEME (Ym base): (per liter) yeast extract (3g); peptone (5g); malt extract (3g); $MgCl_2$"$6H_2O$ (2g)

Ymglu: YEME + glucose (10g)

Ymgal: YEME + galactose (10g)

BACTERIAL STRAINS

In the following Examples, the following strains of *E. coli* are employed:

CGSC Strain #(a)	Strain Designation	Sex	Chromosomal Markers
4473 (*gal*E^{-})	W3109	F^{-}	*galE9*,(b)g^{-};IN(rrnD-rrnE)1
4467 (*gal*T^{-})	W3101	F^{-}	*galT22*(b)g^{-};IN(rrnD-rrnE)1
4498 (*gal*E^{-})	PL-2	Hfr	*thi-1*, *relA1*, *921E28*,g^{-},*spoT1*

(a)CGSC Strain # is the stock number designated for such strain by the *E. coli* Genetic Stock Center of the Department of Human Genetics, Yale University School of Medicine, 333 Cedar Street, P.O. Box 3333, New Haven, Connecticut, 06510, U.S.A.

(b)*galE9* is the old Lederberg *gal9*; *galT22* is the old Lederberg *gal*$_1$.

S1 ANALYSIS

S1 analysis is used to identify the 5' end of RNAs and the length of a RNA of interest. In the following Examples, S1 analysis refers to S1 experiments carried out according to the method of Weaver et al., *Nucl. Acids Res.*, *7*, 1175 (1979) and Berk et al., *Proc. Natl. Acad. Sci. USA*, *75*, 1214 (1978).

EXAMPLE I

A. CLONING OF A *STREPTOMYCES LIVIDANS* GALACTOKINASE GENE.

Streptomyces lividans strain 1326 is described by Bibb et al., *Mol. Gen. Genetics*, *184*, 230-240 (1981) and was obtained from D. A. Hopwood, John Innes Foundation, Norwich, England. *Streptomyces lividans* strain 1326 and *S. lividans* strain 1326 containing the pIJ6 plasmid were deposited in the Agricultural Research Culture Collection, Peoria, Illinois, U.S.A., on June 1, 1982, under accession numbers NRRL 15091 and 15092, respectively.

High molecular weight chromosomal DNA was isolated from Streptomyces lividans strain 1326 according to the method of Maniatis et al., "Molecular Cloning. A Laboratory Manual", Cold Spring Harbor Laboratory (1982) and was size fractionated on a 10-40% sucrose gradient (See, Maniatis et al., cited above, p. 284-285). Fractions of 18-24 kilobase (Kb) pairs were combined and dialyzed exhaustively against 10 mM Tris-HCl/1 mM EDTA (pH 8). Cosmid shuttle vector pJW357 was employed to clone such fractionated chromosomal DNA in its entirety. pJW357 was constructed by fusing pDPT6 cut with PstI to pIJ350 cut with PstI. pIJ350 is described in Kieser et al., Mol. Gen. Genet., 185, 223-238 (1982). pDPT6 is a tetracycline and chloramphenicol resistant, pBR322-based E. coli cosmid cloning vector described in Taylor et al., U.S. Patent No. 4,476,227. pJW357 has a unique EcoRI site in the chloramphenicol resistance gene and a unique BamHI site in the Tc^R (tetracycline) resistance gene. pJW357 was digested with BamHI, dephosphorylated with alkaline phosphatase, and ligated to the fractionated chromosomal DNA described above.

The ligation product was packaged into bacteriophage heads (using the in vitro packaging system described by Maniatis et al., cited above, p. 264-265) and transfected into E. coli strain K21 which is a $galK^-$ derivative of E. coli MM294. The transformation culture was grown for two hours in LB and for an additional two hours in LB with 25 ug/ml chloramphenicol, washed three times with equal volumes of M9 media [see, Miller, "Experiments in Molecular Genetics", Cold Spring Harbor Laboratory (1972)] without a carbon source, and plated onto M9 agar [supplemented with proline, histidine, arginine, isoleucine, leucine, saline and .5% galactose;

See, Adams et al., Biochem. Biophys. Res. Comm., 89(2), 650-58 (1979)] with 30 mg/ml chloramphenicol. Twenty plates were spread with approximately 200 transformants per plate. After three days incubation at 37°C, no transformants were detected. The minimal plates were then sprayed with nicotinic acid to 5 ug/ml to supplement the nicotinic acid requirement of *E. coli* strain K21, and the incubation was continued for 3 more days at 37°C and for 2 additional days at room temperature. After such incubation, the surviving colonies were patched to both MacConkey galactose agar (MAC-GAL) [See, Miller et al., cited above] with 30 ug/ml chloramphenicol and to M63 minimal agar [See, Miller et al., cited above] supplemented with .5% galactose, 5 ug/ml nicotinic acid, 5 ug/ml thiamine and 30 ug/ml chloramphenicol. Only two colonies contained cosmid DNA that transformed *E. coli* K21 to a *gal*K$^+$ phenotype. Such cosmids were designated as pSLIVGAL-1 and pSLIVGAL-2. Both colonies were light red on MAC-GAL (i.e., they were *gal*K$^+$) and also grew on the M63 medium.

Plasmids pSLIVGAL-1 and pSLIVGAL-2 were isolated from the two *gal*K$^+$ colonies described above and were transformed, according to the method of Chater et al., Curr. Top. Micro. Imm., 96, 69-95 (1982), into *Streptomyces lividans* strain 1326-12K (a *gal*K deficient strain isolated after UV mutagenesis of *S. lividans* strain 1326, See, Brawner et al., Gene, 40, 191 (1985), (in press). Plasmid encoded complementation of the *S. lividans* 1326-12K (*gal*K$^-$) host was tested by observing growth of spores plated on MBSM-gal-thiostrepton according to the method of Brawner et al., Gene, 40, 191 (1985) (in press). pSLIVGAL-2 showed no detectable complementation of the *Streptomyces* 1326-12K host.

Cell extracts were prepared from cultures grown in SL medium supplemented with 1% glucose or galactose and 10)g/ml thiostrepton. The extracts were analyzed for galactokinase production by immunoblot analysis (see, Brawner et al., Gene, 40, 191 (1985), in press) using rabbit antisera prepared against E. coli galactokinase. The protein detected by immunoblot analysis was the approximate size of E. coli galK. Such protein appeared in galactose supplemented cultures of Streptomyces at levels several fold higher than in glucose cultures.

B. MAPPING OF THE S. LIVIDANS GALK REGION WITHIN A COSMID.

The galK region of the pSLIVGAL1 and pSLIVGAL2 cosmids, prepared as described above, was identified by cloning random fragments from the cosmids into a pUC18 derivative [See, Norrander et al., Gene, 26, 101-106 (1983)] and scoring complementation of E. coli strain MM294 ($galK^-$) on MAC-GAL medium. The cosmid clone was partially digested with Sau3AI (using conditions which maximized the yield of 2 to 4 kilobase fragments), and the products of this reaction were ligated into the BglII site of pUC18-TT6, a derivative of pUC18 constructed by insertion of the following synthetic DNA sequence into the BamHI site of pUC18:

```
5'GATCAGATCTTGATCACTAGCTAGCTAG 3'
3'    TCTAGAACTAGTGATCGATCGATCCTAG 5'
```

Twelve $galK^+$ clones (red on MAC-GAL) were screened for size. One clone, designated as plasmid pSAU10, was the smallest and had an insert size of approximately 1.4 Kb.

In contrast to colonies containing pSLIVGAL1, the pUC clones were very red on MAC-GAL medium, indicating an increased production of galactokinase. The most likely explanation for the increased enzyme level was that the S. lividans galK gene was now being transcribed by an E. coli promoter which was stronger than the upstream promoter on the cosmid.

The insert of pSAU10 was isolated as an EcoRI to HindIII fragment (these sites flank the insert region of pUC18-TT6) for use as a probe for the *S. lividans galK* gene. The chromosomal DNA used in the cloning was restricted with EcoRI plus MluI and BamHI plus BglII, and then blotted according to the method of Southern, *J. Mol. Biol.*, 98, 503 (1975). The pSAU10 fragment was nick translated and hybridized to the blot. The probe identified a 1.3 kb EcoRI-MluI fragment and a 5 kb BamHI-BglII fragment in the chromosomal digests. When this data was compared to the map of the cosmid insert, the location of the *galK* gene (between map positions 5 and 7, See Table A) was confirmed.

C. DNA SEQUENCING OF THE *S. LIVIDANS GAL* OPERON.

The *Streptomyces lividans gal* operon was sequenced by chain termination [(See, Sanger et al., *Proc. Nat'l Acad. Sci., U.S.A.*, 74, 5463 (1977)] and chemical cleavage [See, Maxam and Gilbert, *Methods in Enzymology*, 65, 499 (1980)]. The initial sequences of *galK* were derived from Sau3AI and SalI fragments of the insert of pSAU6 (a 2.3 Kb sibling of pSAU10) shotgun cloned into the BamHI and SalI sites (respectively) of M13 mp 10 [See, Messing, *Methods in Enzymology*, 101, 20 (1983)]. Amino acid sequences of the *S. lividans galT*, *galE* and *galK* genes were predicted by computer, and further analyzed by comparison with amino acid sequences of the *E. coli* and or *S. cerevisiae* galactokinase, gal-1-phosphate uridyltransferase and UDP-4-epimerase enzymes. The sequences of these proteins were predicted by computer analysis using the total or partial DNA sequence of the genes which encode the *gal* enzymes [see, Debouck et al., *Nuc Acids. Res.*, 13(6), 1841-1853 (1985), and Citron and Donelson, *J. Bacteriology*, 158, 269 (1984)]. Some homology was found between the inferred protein sequence for the S. lividans galK, galT, galE gene products and their respective E. coli and/or S. cerevisiae gene products.

The complete DNA sequence of the S. lividans gal operon is shown in Table 1. Included in Table 1 are the transcription start sites for the operon's promoters and the predicted amino acid sequences of the galT, galE and galK gene products.

TABLE 1

TRANSLATED SEQUENCE OF *STREPTOMYCES LIVIDANS* GALACTOSE OPERON

```
      -120         -110         -100           -90          -80          -70
        .            .            .             .            .            .
CTA CGC CTC CGC GTT CAG TAA TTG AAC ACT TTT GGT GAT GAA CTT TGT TTG ATT GTC


           -60          -50          -40           -30          -20
            .            .            .             .            .
ATG TGA CAG GGG GGT GGT GGG TTG TGA TGT GTT ATG TTT GAT TGT GTT GGA TGA TTG
                                                           ^galP1

-10             1          10           20           30            40
  .             .           .            .            .             .
ACG GGC GTC CTG GTG ACT CAT GGG TGG GTG CAG AGG AGT GCG GCA GTG AAG AAG ACC
                Met Thr His Gly Trp Val Gln Arg Ser Ala Ala Val Lys Lys Thr
                galT
    50           60            70           80           90            100
     .            .             .            .            .             .
TCG ACC CGG CTG GCC GAC GGC CGT GAG CTG GTC TAC TAC GAC CTG CGC GAC GAC ACC
Ser Thr Arg Leu Ala Asp Gly Arg Glu Leu Val Tyr Tyr Asp Leu Arg Asp Asp Thr

        110          120            130          140          150
         .            .             .            .            .
GTG CGC GAC GCC GTG GAC CGC CGT CCG CTG GAG CGG ACC GTC ACC ACG TCC GAG GTG
Val Arg Asp Ala Val Asp Arg Arg Pro Leu Glu Arg Thr Val Thr Thr Ser Glu Val

160         170          180           190          200          210
.            .            .             .            .            .
CGA CGC GAC CCG CTG CTC GGC GAC TCC GCG CCG TCG CGC CTC GCA CCG GCA GGG GCG
Arg Arg Asp Pro Leu Leu Gly Asp Ser Ala Pro Ser Arg Leu Ala Pro Ala Gly Ala

   220          230          240           250          260          270
    .            .            .             .            .            .
CAC CTA CCA TCC GCC GGC CGA CCA GTG CCC GCT GTG CCc GTC GGA CGG GGA ACG GCT
His Leu Pro Ser Ala Gly Arg Pro Val Pro Ala Val Pro Val Gly Arg Gly Thr Ala

       280          290          300           310          320          330
        .            .            .             .            .            .
GAG CGA GAT CCG GCC TAT GAC GTG GTG GTC TTC GAG AAT CGC TTT CCC TCG CTG GCC
Glu Arg Asp Pro Ala Tyr Asp Val Val Val Phe Glu Asn Arg Phe Pro Ser Leu Ala
```

Table 1 - (cont'd)

```
           340          350          360           370          380
            .            .            .             .            .
GGT GAC TCC GGG CGC TGC GAG GTC GTC TGC TTC ACC TCC GAC CAC GAC GCC TCC TTC
Gly Asp Ser Gly Arg Cys Glu Val Val Cys Phe Thr Ser Asp His Asp Ala Ser Phe

 390           400          410          420           430          440
  .             .            .            .             .            .
GCC GAC CTG AGC GAG GAG CAG GCC CGG CTG GTC GTC GAC GCC TGG ACG GAC CGC ACC
Ala Asp Leu Ser Glu Glu Gln Ala Arg Leu Val Val Asp Ala Trp Thr Asp Arg Thr

     450           460          470          480           490          500
      .             .            .            .             .            .
TCC GAG CTG TCC CAT CTG CCC TCC GTT GAA CAG GTG TTC TGC TTC GAG AAC CGG GGC
Ser Glu Leu Ser His Leu Pro Ser Val Glu Gln Val Phe Cys Phe Glu Asn Arg Gly

         510           520          530          540           550
          .             .            .            .             .
GCC GAG ATC GGG GTG ACG CTG GGT CAC CCG CAC GGG CAG ATC TAC GCC TAC CCG TTC
Ala Glu Ile Gly Val Thr Leu Gly His Pro His Gly Gln Ile Tyr Ala Tyr Pro Phe

560          570           580          590          600           610
 .            .             .            .            .             .
ACC ACC CCC CGC ACC GCC CTG ATG CTC CGT TCA CTC GCC GCC CAC AAG GAC GCG ACG
Thr Thr Pro Arg Thr Ala Leu Met Leu Arg Ser Leu Ala Ala His Lys Asp Ala Thr

    620          630           640          650          660           670
     .            .             .            .            .             .
GGC GGG GGG AAC CTG TTC GAC TCC GTG CTG GAG GAG GAG CTG GCC GGT GAG CGG GTC
Gly Gly Gly Asn Leu Phe Asp Ser Val Leu Glu Glu Glu Leu Ala Gly Glu Arg Val

        680          690           700          710          720
         .            .             .            .            .
GTC CTG GAG GGT GAG CAC TGG GCC GCC TTC GTC GCG TAC GGC GCG CAC TGG CCG TAC
Val Leu Glu Gly Glu His Trp Ala Ala Phe Val Ala Tyr Gly Ala His Trp Pro Tyr

730         740          750           760          770          780
.            .            .             .            .            .
GAG GTG CAC CTC TAC CCG AAG CGG CGG GTG CCC GAT CTG CTC GGG CTC GAC GAG GCG
Glu Val His Leu Tyr Pro Lys Arg Arg Val Pro Asp Leu Leu Gly Leu Asp Glu Ala

   790          800          810           820          830          840
    .            .            .             .            .            .
GCT CGC ACA GAA TTC CCC AAG GTC TAC CTG GAG CTG CTG AGG CGT TTC GAC CGG ATC
Ala Arg Thr Glu Phe Pro Lys Val Tyr Leu Glu Leu Leu Arg Arg Phe Asp Arg Ile
```

Table 1 - (cont'd)

```
        850       860       870       880       890       900
         .         .         .         .         .         .
TTC GGC GAG GGC GAG CCC CCG ACC CCC TAC ATC GCG GCC TGG CAC CAG GCG CCG TTC
Phe Gly Glu Gly Glu Pro Pro Thr Pro Tyr Ile Ala Ala Trp His Gln Ala Pro Phe

          910       920       930       940       950
           .         .         .         .         .
GGC CAG CTG GAG TTC GAG GGT GTG ACG CGC GAC GAC TTC GCG CTC CAC CTG GAA CTT
Gly Gln Leu Glu Phe Glu Gly Val Thr Arg Asp Asp Phe Ala Leu His Leu Glu Leu

 960       970       980       990       1000      1010
  .         .         .         .         .         .
TTC ACT TCC GCC GTA CGT CCG GCA AGC TGA AGT TCC TCG CGG GCT CCG AAT CCG GCA
Phe Thr Ser Ala Val Arg Pro Ala Ser ---             ^galP2

      1020      1030      1040      1050      1060      1070
        .         .         .         .         .         .
  TGAACG TGTTCATCAA CGACGTACCC CCGGAGCGCG CGGCCGAGCG ACTGCGAGAG GTAGCGAG

1080       1090       1100       1110       1120       1130
  .          .          .          .          .          .
TTC ATG AGC GGG AAG TAC CTG GTG ACA GGT GGT GCC GGA TAC GTC GGC AGC GTC GTC
    Met Ser Gly Lys Tyr Leu Val Thr Gly Gly Ala Gly Tyr Val Gly Ser Val Val
    galE
    1140      1150       1160      1170      1180       1190
     .         .          .         .         .          .
GCC CAG CAC TTG GTG GAG GCG GGG AAC GAG GTC GTG GTG CTG CAC AAT CTG TCG ACC
Ala Gln His Leu Val Glu Ala Gly Asn Glu Val Val Val Leu His Asn Leu Ser Thr

        1200      1210       1220      1230      1240
          .         .          .         .         .
GGC TTC CGT GAG GTG TGC CGG CGG GTG CCT CGT TCG TCG AGG CGA CAT CCG GGA CGC
Gly Phe Arg Glu Val Cys Arg Arg Val Pro Arg Ser Ser Arg Arg His Pro Gly Arg

1250      1260      1270       1280      1290      1300
 .         .         .          .         .         .
CGC CAA GTG CGT GGA CGG CTC TCG TTC GAC GGC GTG CTG CAC TTC GCC GCC TTC TCC
Arg Gln Val Arg Gly Arg Leu Ser Phe Asp Gly Val Leu His Phe Ala Ala Phe Ser

   1310      1320      1330       1340      1350      1360
    .         .         .          .         .         .
CAG GTC GGC GAG TCG GTC GTG AAG CCC GAG AAG TAC TGG GAC AAC AAC GTC GGT GGC
Gln Val Gly Glu Ser Val Val Lys Pro Glu Lys Tyr Trp Asp Asn Asn Val Gly Gly
```

Table 1 - (cont'd)

```
      1370         1380         1390          1400         1410         1420
        .            .            .             .            .            .
ACC ATG GCG CTG CTG GAG GCC ATG CGG GGC GCG GGT GTG CGG CGG CTC GTC TTC TCC
Thr Met Ala Leu Leu Glu Ala Met Arg Gly Ala Gly Val Arg Arg Leu Val Phe Ser

          1430         1440         1450          1460         1470
            .            .            .             .            .
TCC ACG GCC GCC ACG TAC GGC GAG CCC GAG CAG GTT CCC ATC GTC GAG TCC GCG CCG
Ser Thr Ala Ala Thr Tyr Gly Glu Pro Glu Gln Val Pro Ile Val Glu Ser Ala Pro

1480          1490         1500         1510          1520         1530
  .             .            .            .             .            .
ACG AGG CCC ACC AAT CCG TAC GGC GCC TCG AAG CTC GCC GTC GAC CAC ATG ATC ACC
Thr Arg Pro Thr Asn Pro Tyr Gly Ala Ser Lys Leu Ala Val Asp His Met Ile Thr

    1540          1550         1560         1570          1580         1590
      .             .            .            .             .            .
GGC GAG GCG GCG GCC CAC GGG CTG GGC GCG GTC TCC GTG CCG TAC TTC AAC GTC GCG
Gly Glu Ala Ala Ala His Gly Leu Gly Ala Val Ser Val Pro Tyr Phe Asn Val Ala

        1600          1610         1620         1630          1640
          .             .            .            .             .
GGC GCG TAC GGG GAG TAC GGC GAG CGC CAC GAC CCC GAG TCG CAT CTG ATT CCG CTG
Gly Ala Tyr Gly Glu Tyr Gly Glu Arg His Asp Pro Glu Ser His Leu Ile Pro Leu

1650        1660          1670         1680         1690          1700
 .            .             .            .            .             .
GTC CTT CAA GTG GCG CAG GGC AGG CGG GAG GCC ATC TCC GTC TAC GGC GAC GAC TAC
Val Leu Gln Val Ala Gln Gly Arg Arg Glu Ala Ile Ser Val Tyr Gly Asp Asp Tyr

   1710         1720          1730         1740         1750          1760
     .            .             .            .            .             .
CCG ACG CCG GAC CGA CCT GTG TGC GCG ACT ACA TCC ACG TCG CCG ACC TGG CCG AGG
Pro Thr Pro Asp Arg Pro Val Cys Ala Thr Thr Ser Thr Ser Pro Thr Trp Pro Arg

       1770         1780          1790         1800         1810
         .            .             .            .            .
CCC ACC TGC TGG CCG TGC GCC GCC GCC CCG GGC GAG CAC CTC ATC TGC AAC CTG GGC
Pro Thr Cys Trp Pro Cys Ala Ala Ala Pro Gly Glu His Leu Ile Cys Asn Leu Gly

1820       1830         1840          1850         1860         1870
.            .            .             .            .            .
AAC GGC AAC GGC TTC TCC GTC CGC GAG GTC GTC GAG ACC GTG CGG CGG GTG ACG GGC
Asn Gly Asn Gly Phe Ser Val Arg Glu Val Val Glu Thr Val Arg Arg Val Thr Gly
```

Table 1 - (cont'd)

```
   1880         1890         1900          1910         1920         1930
     .            .            .             .            .            .
CAT CCG ATC CCC GAG ATC ATG GCC CCG CGC CGC GGG CGC GAC CCG GCG GTC CTG GTC
His Pro Ile Pro Glu Ile Met Ala Pro Arg Arg Gly Arg Asp Pro Ala Val Leu Val

       1940         1950         1960          1970         1980        1990
         .            .            .             .            .           .
GCG TCC GCC GGC ACC GCC CGC GAG AAG CTG GGC TGG AAC CCG TCC CGC GCG GAC CTC
Ala Ser Ala Gly Thr Ala Arg Glu Lys Leu Gly Trp Asn Pro Ser Arg Ala Asp Leu

           2000         2010         2020          2030         2040
             .            .            .             .            .
GCC ATC GTG TCG GAC GCG TGG GAG TTG CCG CAG CGG CGC GCG GGC CAG TAG  TA
Ala Ile Val Ser Asp Ala Trp Glu Leu Pro Gln Arg Arg Ala Gly Gln ---


       2050         2060         2070          2080         2090        2100
         .            .            .             .            .           .
ACC GCA GTT ACC GGA AAG GCG ACG GGT CAG GGC ATG GGC GAG GCT GTC GGG GAA CCG
                                            Met Gly Glu Ala Val Gly Glu Pro
                                            galK
           2110         2120         2130          2140         2150
             .            .            .             .            .
TCG GCC AGC GGT TCC GGG AGC TGT ACG GGC CGG AGC CGG AGG GGG TGT GGG CGC CGA
Ser Ala Ser Gly Ser Gly Ser Cys Thr Gly Arg Ser Arg Arg Gly Cys Gly Arg Arg

 2160          2170         2180         2190          2200         2210
   .             .            .            .             .            .
GCG GGC CGG GAG AAC CTC ATC GGG GAG CAC ACC GAC TAC AAC GAC GGC TTC GTC ATG
Ala Gly Arg Glu Asn Leu Ile Gly Glu His Thr Asp Tyr Asn Asp Gly Phe Val Met

     2220          2230         2240         2250          2260         2270
       .             .            .            .             .            .
CCT TCG CCC TGC CGC ACC AGG TCG CGG CCG TCT CCC GGC GCG AAC GAC GGC ATC CTG
Pro Ser Pro Cys Arg Thr Arg Ser Arg Pro Ser Pro Gly Ala Asn Asp Gly Ile Leu

         2280          2290         2300         2310          2320
           .             .            .            .             .
CGC CTG CAC TCG GCC GAC GTC GAC GCC GAC CCG GTC GAG CTG CGC GTC GCC GAC CTG
Arg Leu His Ser Ala Asp Val Asp Ala Asp Pro Val Glu Leu Arg Val Ala Asp Leu

2330         2340          2350         2360         2370          2380
  .            .             .            .            .             .
GCC CCC GCG TCG GAC AAG TCC TGG ACG GCG TAC CCC TCG GGC GTC CTG TGG GCG CTG
Ala Pro Ala Ser Asp Lys Ser Trp Thr Ala Tyr Pro Ser Gly Val Leu Trp Ala Leu
```

Table 1 - (cont'd)

```
  2390       2400       2410       2420       2430       2440
CGC GAG GCC GGA CAC GAG CTG ACC GGC GCC GAC GTC CAC CTG GCC TCC ACC GTC CCG
Arg Glu Ala Gly His Glu Leu Thr Gly Ala Asp Val His Leu Ala Ser Thr Val Pro

       2450       2460       2470       2480       2490
TCC GGG GCG GGC CTC TCC TCC TCC GCG GCC CTG GAG GTC CGT CCC CTG GCG ATG AAC
Ser Gly Ala Gly Leu Ser Ser Ser Ala Ala Leu Glu Val Arg Pro Leu Ala Met Asn

2500       2510       2520       2530       2540       2550
GAC CTG TAC GCC CTC GCG CTG CGC GGC TGG CAG CTG GCC CGG CTG TGC CAG CGC GCG
Asp Leu Tyr Ala Leu Ala Leu Arg Gly Trp Gln Leu Ala Arg Leu Cys Gln Arg Ala

   2560       2570       2580       2590       2600       2610
GAG AAC GTC TAC GTC GGC GCC CCC GTC GGC ATC ATG GAC CAG ACG GCG TCC GCC TGC
Glu Asn Val Tyr Val Gly Ala Pro Val Gly Ile Met Asp Gln Thr Ala Ser Ala Cys

       2620       2630       2640       2650       2660       2670
TGC GAG GCG GGC ACG CCC TCT TCC TCG ACA CCC GCG ACC TCT CCC AGC GGC AGA TCC
Cys Glu Ala Gly Thr Pro Ser Ser Ser Thr Pro Ala Thr Ser Pro Ser Gly Arg Ser

          2680       2690       2700       2710       2720
CCT TCG ACC TCG CCG CCG AGG GGA TGC GCC TGC TGG TCG TCG ACA CCC GGG TCA AGC
Pro Ser Thr Ser Pro Pro Arg Gly Cys Ala Cys Trp Ser Ser Thr Pro Gly Ser Ser

2730       2740       2750       2760       2770       2780
ACT CCC ACA GCG AGC GCG AGT ACG GCA AGC GCC GCG CGG GCT GCG AGA AGG GCG CCG
Thr Pro Thr Ala Arg Ala Ser Thr Ala Ser Ala Ala Arg Ala Ala Arg Arg Ala Pro

    2790       2800       2810       2820       2830       2840
CGC TGC TGG GCG TCG ACG CGC TGC GAC GTG CCG TAC GCC GAC CTG GAC GCG GCG CTG
Arg Cys Trp Ala Ser Thr Arg Cys Asp Val Pro Tyr Ala Asp Leu Asp Ala Ala Leu

         2850       2860       2870       2880       2890
GAG CGG CTC GGC GAC GAG GAG GAG GTG CGC CGC CTG GTC CGG CAC GTG GTG ACC GAG
Glu Arg Leu Gly Asp Glu Glu Glu Val Arg Arg Leu Val Arg His Val Val Thr Glu
```

Table 1 - (cont'd)

```
2900         2910          2920         2930         2940          2950
 •            •             •            •            •             •
GAC GAG CGC GTC GAA CGG GTG GTC GCC CTG CTG GAG TCG GCG ACA CCC GGC GCA TCG
Asp Glu Arg Val Glu Arg Val Val Ala Leu Leu Glu Ser Ala Thr Pro Gly Ala Ser

    2960         2970          2980         2990         3000          3010
     •            •             •            •            •             •
GCC CCG TCC TGG TCG AGG GCC ACG CCT GCT GCG CGA CGA CTT CCG CAT CTC CTG CCC
Ala Pro Ser Trp Ser Arg Ala Thr Pro Ala Ala Arg Arg Leu Pro His Leu Leu Pro

        3020         3030          3040         3050         3060
         •            •             •            •            •
CGA GCT GGA CCT GGT CGT CGA CAC GGC CCT GGC CTC CGC GGC CCT CGG CGC CGG ATG
Arg Ala Gly Pro Gly Arg Arg His Gly Pro Gly Leu Arg Gly Pro Arg Arg Arg Met

3070        3080         3090          3100         3110         3120
•            •            •             •            •            •
ACC GGC GGC GGC TTC GGC GGC TCG GCG ATC GTC CTG GTG GAG GCC GCC GCG GTG GAC
Thr Gly Gly Gly Phe Gly Gly Ser Ala Ile Val Leu Val Glu Ala Ala Ala Val Asp

   3130         3140         3150          3160         3170         3180
    •            •            •             •            •            •
GCC GTC ACC AAG GCG GTC GAG GAC GCC TTC GCC GCG GCG GGC CTC AAG CGT CCG CGG
Ala Val Thr Lys Ala Val Glu Asp Ala Phe Ala Ala Ala Gly Leu Lys Arg Pro Arg

       3190         3200         3210          3220         3230         3240
        •            •            •             •            •            •
GTG TTC GAG GCG GTG CCT CGG CGG GGC GCG GCG CCT GGT CTG ACG GTC AGC CGA GCC
Val Phe Glu Ala Val Pro Arg Arg Gly Ala Ala Pro Gly Leu Thr Val Ser Arg Ala

           3250         3260         3270          3280         3290
            •            •            •             •            •
GCT TCA CCA GCG TGT ACT CCG TGA TCC CCG GCG GGT AGT CGG GGA TCA CGC ACA TGA
Ala Ser Pro Ala Cys Thr Pro ---

 3300
  •
GCT GCT AGC CGC
```

EXAMPLE 2

PROMOTERS OF THE S. LIVIDANS GAL OPERON

a) P1 promoter

(i) Summary

This promoter is galactose inducible, glucose repressible and is the regulatable promoter for the entire Streptomyces gal operon. S1 data indicates that the Streptomyces lividans gal operon encodes a polycistronic transcript of approximately 3.4 kilobases (Kb). The transcript consists of approximately 1 Kb for galT, followed by approximately 1 Kb each for galE and galK. (See, Figure 1).

Galactose induction of P1 is mediated, at least in part, by an operator sequence whose 5' end is located 31 bp upstream of the transcription start site and a repressor protein which recognizes the operator.

(ii) Experimental: Isolation, Localization, and Characterization of the P1 promoter.

The sequences upstream of the Streptomyces lividans galK ATG were screened for promoters using the E. coli galK promoter probe system of Brawner, et al., Gene, 40, 191, (1985), in press. The HindIII-MluI fragment (See, Table A, map positions 1-5) was restricted with Sau3AI, ligated into the unique BamHI site of pK21 (Figure 2), and transformed into E. coli K21 (galK$^-$) according to the method of Example 1. pK21 is a derivative of pSKO3 and is an E. coli-Streptomyces shuttle vector containing the E. coli galK gene (See, Figure 2). The construction of pSKO3 is described in Rosenberg et al., Genetic Engineering, 8, (1986), in press. The clones which expressed galK, i.e., those which had promoter activity, were identified on MacConkey - galactose plates. Two galK$^+$ clones (designated as pK21 MH1 and 2) were transformed into Streptomyces 1326-12K (galK$^-$).

Extracts from transformants were cultured in Ymglu and Ymgal, and were analyzed by western blot analysis using anti-E. coli galactokinase antiserum. The blots showed significantly higher levels of galactokinase in the extracts from the galactose induced cultures.

pK21 MH1 and 2 were shown by restriction analysis to contain a 410 bp Sau3AI insert which is contained within the HindIII and BglII sites (see Table A, map positions 1-2) by Southern blot analysis according to the method of Southern, J. Mol. Biol., 98, 503 (1975). The cloned fragment was analyzed by S1 analysis using RNA isolated from Streptomyces lividans 1326-12K and E. coli K21 cultures. The fragment yielded a 290 nucleotide protected fragment after S1 digestion (indicating the 5' end of an mRNA 290 bp upstream of the Sau3AI site). Hybridization experiments (using single stranded M13 clones of this region) have identified the direction of transcription as left to right as shown in Figure 2 (i.e., transcription is going toward galK).

Conventional DNA sequence analysis and additional S1 mapping analysis were used to define the 5'end of the mRNA.

The sequences responsible for regulating galactose induction of P1 were localized by removing sequences upstream of the transcription start site by nuclease Bal31. Any change in promoter function or galactose induction by removal of these sequences was assessed using the E. coli galK promoter probe plasmid used to identify P1.

(iii) Construction of Gal Promoter Deletions.

Plasmid pHL5 was constructed by cloning a DNA fragment containing 100 bp of sequences downstream from the start of P1 transcription and 216 bp upstream from the start of P1 transcription into plasmid pUC19TT1. Plasmid pUC19TT1 is described in Norrander et al., Gene, 26, 101-106 (1983) and has the Unker as pUC18-TT6. See, Example IB. Deletions extending into the upstream sequence preceeding P1 were generated by linearizing pHL5 with HindIII and treating the ends with nuclease Bal31. The uneven ends were subsequently repaired with the Klenow fragment of DNA polymerase I. Bal31-treated pHL5 was then digested with BamHI and run on a 5% acrylamide gel. DNA fragments in the molecular weight range of 100-300 bp were eluted from the gel and subcloned into M13 mp 10 that had been digested with HindII and BamHI. [See, Messing, Methods in Enzymology, 101, 20 (1983)]. Individual deletions were then sequenced from the single stranded phage DNA the dideoxy chain termination method of Sanger, et al., cited above.

(iv) Linking the P1 Promoter Deletions to the E. coli galK Gene.

The various mp 10 clones were digested with BamHI and HindIII. DNA fragments containing individual deletions were isolated from low-melting point agarose gels and then ligated to pK21 (see, Figure 2) that had been digested with BamHI and HindIII. After transformation into E. coli MM294, plasmid DNA was isolated for each of the deletion derivatives and transformed into Streptomyces Lividans 12K.

(v) Functional Assessment of Bal 31-Generated Deletions in S. lividans

For each individual promoter deletion, a single thiostrepton resistant transformant was grown to late log in YM base (YEME) + 10 ug/ml thiostrepton. Cells were then pelleted, washed once in M56 media and resuspended in M56 media (see Miller, et al., cited above). The washed cells were then used to inoculate YM + 0 1M MOPS (pH 7.2) + 10 ug/ml thiostrepton supplemented with 1% galactose or 1% glucose. The cells were grown for 16 hours then assayed for galactokinase activity.

Ten individual pK21 derivatives containing either 120, 67, 55, 34, 31, 24, 20, 18, 10 or 8 bp of sequence upstream of the P1 transcription start site were analyzed for galactokinase expression. These results showed that all the information necessary for galactose induction of Pl, (i.e., 10-20 fold greater levels of galactokinase produced in galactose grown cells versus glucose grown cells) is included in the 31 bp of sequence upstream of Pl. A deletion which leaves 34 bp of sequence upstream of Pl is partially inducible by galactose since galactose induced 6-fold greater amounts of galactokinase. Thus, one end of the operator must be situated within the sequences between the -24 and -31 position. The remaining deletions which leave either 20, 18, 10 or 8 bp of upstream sequence result in a constitutive Pl promoter, that is the levels of galactokinase produced were equivalent when cells were grown in the presence of galactose or glucose. Although the promoter deletions which retained 8 and 10 bp of Pl were constitutive, the amount of galactokinase produced was reduced 10 fold in comparison to the promoter deletions which retained 18 to 120 bp of upstream sequence. This result indicates that sequences between the -10 and -18 positions of -1 are essential for promoter function.

This data supports a model in which galactose induction of Pl is mediated, at least in part, by an operator sequence. One end of this sequence is 24 to 31 bp upstream of the Pl transcription start site. Removing part or all of the operator results in a promoter which is partially or totally derepressed. The other end of this sequence has not been defined by these experiments but it most likely is contained within the 24 to 31 bp of sequence upstream of the Pl transcription start site. In addition we cannot eliminate the possibility that the 3' end of the operator is also within the 100 bp downstream of the transcription start site since these sequences were contained within the smallest region needed to achieve galactose induction. These data also suggest that the factor which interacts with the operator sequence is a repressor protein. Finally, we do not have any evidence which eliminates the possibility that P1 may be controlled by factors other than a repressor (i.e., positive activator such as lambda phage cII protein) to modulate galactose induction promoter transcript.

b) P2 promoter

(i) Summary

The P2 promoter of the Streptomyces gal operon is upstream of the galE gene and transcribes both galE and galK genes.

P2 promoter expression is constitutive (i.e., not glucose repressed/galactose induced) as shown by S1 analysis.

(ii) Experimental: Isolation, Localization, and Characterization of the P2 promoter.

The existence of the Streptomyces gal operon P2 promoter became apparent when the BglII-MluI fragment (see, Table A, map positions 2-5) of S. lividans 1326 DNA was inserted into plasmid pK21 (see, Figure 2) and galactokinase expression was observed in Streptomyces lividans 1326-12K transformed therewith.

DNA sequence analysis and S1 analysis were used to identify the 5' end of the S. lividans gal operon P2. The 5' end of the P2 promoter transcript is within 100 bp upstream of the predicted galE ATG.

EXAMPLE 3

EVIDENCE OF A POLYCISTRONIC MESSAGE IN THE STREPTOMYCES GAL OPERON

S1 analysis was used to map the transcripts upstream and downstream of the Streptomyces lividans gal operon galK gene. In general, overlapping DNA fragments of 1-2 Kb were isolated from subclones, further restricted, and end labelled. The message was followed from the 3' end of galK to the upstream end at P1.

The 3' end of the Streptomyces lividans gal operon transcript probably occurs within the first hundred bases downstream of galK. Fragments 3' labelled at sites within the galK sequence were not protected to their full length (S1 analysis) if they extend into this downstream region. One experiment showed a possible protected region that terminated 50-100 bp downstream of the galK translation stop. The existence of a transcription terminator can be confirmed by conventional techniques by using a terminator probe system. The gal operon transcript clearly does not extend to the PvuII site (see, Table A, map position 8) because no full length protection of 5' labelled PvuII fragments occurs from that site.

5' end labelled fragments from two PvuII fragments, fragment I, (map positions 4-6, See, Table A), and fragment II, (map positions 6-8, See Table A), and the insert of pSau10 were used as sources of probes for S1 walking from the 3' to 5' end of the message. All fragments through this region are protected, except the fragment containing the P2 promoter which shows partial and full protection. The complete protection from S1 digest indicates a polycistronic message which initiates upstream at P1 and continues to approximately 100 bp downstream of galK.

The above data is indirect evidence of a polycistronic mRNA of the Streptomyces gal operon. S1 analysis using a long contiguous DNA fragment (e.g., the 4.5 kb HindIII-SacI fragment, see map position 7 of Table A) has been used to confirm the transcript size.

EXAMPLE 4

LOCALIZATION OF S. LIVIDANS GAL OPERON GALE AND GALT GENES

(i) Summary

The S. lividans gal operon galE gene was localized to 1.5 Kb PvuII fragment (map position, 4-6 of Table A) of pLIVGAL1 (Figure 1).

The S. lividans gal operon galE coding sequences extend through the MluI site (map position 5 of Table A).

The S. lividans gal operon galT gene was localized within the 1.15 Kb Nru-PvuII region (see, Table A, map positions 1a-4) of pSLIVGAL1.

The direction of S. lividans gal operon galE and galT transcription is the same as galK gene.

(ii) Experimental

It was necessary to identify the other functions contained on pLIVGAL1; specifically, does this plasmid encode for the enzyme galactose epimerase (galE) or the enzyme galactose transferase (galT). The Streptomyces gal operon galK gene was identified by its ability to complement an E. coli galK host. Thus, identification of the Streptomyces galT and galE genes was tested for by complementation of E. coli galE$^-$ or galT$^-$ hosts, respectively. An E. coli galT$^-$ strain (CGSC strain #4467, W3101) and two galE$^-$ strains (CGSC strain #4473; W3109 and CGSC strain #4498; PL-2) were obtained to test for complementation by the pSLIVGAL1 clone.

The ca. 9 Kb HindIII-SphI fragment (see, Table A, map positions 1-16) containing the Streptomyces lividans gal operon galK gene was inserted into pUC19. This fragment was situated within pUC19 such that transcription from the Plac promoter of pUC19 is in the same direction as the Streptomyces galK gene. pUC19 is described in Yanisch-Perrou, et al., Gene, 33, 103 (1983). Complementation was assayed by growth on MacConkey-galactose plates. Cells which can utilize galactose [galE$^+$, galT$^+$, galK$^+$] will be red to pink on this medium. *E. coli* strain PL-2 (see, Example 2) containing pUC19 with the *Hind*III-*Sph*I insert were pink on the indicator plate indicating that the *Hind*III-*Sph*I fragment contains the *Streptomyces lividans* *gal*E gene. The *gal*E gene was later mapped to within the 4.5 Kb *Hind*III-*Sac*I (the *Sac*I site is near the region around map position 7-8 of Table A) fragment. If the sequences from the *Mlu*I site (map position 5 of Table A) to the *Sac*I site were removed *gal*E complementation of *E. coli* PL-2 was not detected. The 5' end of the *gal*K gene is 70 base pairs (bp) from the *Mlu*I site. Therefore it seemed likely that the *Mlu*I site was contained within the 5' or 3' end of the *gal*E gene. To determine the direction of *gal*E transcription, the *Hind*III-*Sac*I fragment was inserted into pUC18. In this configuration, the *Streptomyces lividans* *gal*K gene is in the opposite orientation with respect to P*lac*. The pUC18 *Hind*III-*Sph*I clone did not complement *E. coli* PL-2 indicating the *gal*E is transcribed in the same direction as *gal*K. In addition it was concluded that the *Mlu*I site is contained within the 3' end of the *gal*E gene. DNA sequence analysis of the *Pvu*II-*Mlu*I fragment (See, Table A, map position 4-5) has identified an open reading frame which encodes for a polypeptide of predicted molecular weight of 33,000 daltons. The 5' end of this reading frame is located approximately 176 bp from the *Pvu*II site (See, Table A, map position 4). Therefore, the sequencing results support the conclusion that the 3' end of *gal*E traverses the *Mlu*I site (see, Table A, map position 5).

Similar experiments to localize the *gal*T gene on pSLIVGAL1 were attempted with the *gal*T hosts.

The region between P1 and the 5' end of *gal*E was sequenced to identify the *gal*T gene. Translation of the DNA sequence to the amino acid sequence identified a reading frame which encodes a protein showing a region of homology to the yeast transferase.

EXAMPLE 5

GALACTOSE INDUCTION OF S. LIVIDANS GAL OPERON GALK GENE

(i) Summary

Galactokinase expression is induced within one hour after the addition of galactose to culture medium.

Galactokinase expression is 10 times higher in the presence of galactose versus glucose or no additional carbon source within 6 hours after addition of the sugar.

(ii) Experimental

Galactose induction of the *Streptomyces lividans galK* gene was examined by assaying for galactokinase activity at 1, 3, 6 and 24 hours after the addition of galactose. Two liters of YM + 0.1M MOPS (pH 7.2) were inoculated with 2×10^7 spores of *Streptomyces lividans* 1326. After 21 hours growth, galactose or glucose were added to a final concentration of 1%. One, three, six and twenty four hours after the addition of sugar, cells were isolated and assayed for galactokinase activity. Total RNA was prepared by procedures described in Hopwood et al., cited above.

An increase in galactokinase synthesis was observed one hour after the addition of galactose. The increase continued over time (1 to 24 hours). S1 analysis of RNA isolated from the induced cultures confirmed that the increase in *galK* activity was due to increased levels of the P1 promoter transcript.

The S1 data and the induction studies suggest the following model for gene expression within the *Streptomyces gal* operon. The P1 promoter is the galactose inducible promoter. The P1 transcript includes *galT*, *galE* and *galK*. The P2 promoter is constitutive and its transcript includes *galE* and *galK*.

It is interesting to note that the E. coli gal operon also has two promoters, P1 and P2. [See, Nusso et al., Cell, 12, 847 (1977)]. P1 is activated by cAMP-CRP binding whereas P2 is inhibited by cAMP-CRP. Translation of the E. coli gal operon galE coding sequence is more efficient when transcription initiates at P2 which serves to supply a constant source of epimerase even in the absence of galactose or the presence of glucose [See, Queen et al., Cell, 25, 241 (1981)]. The epimerase functions to convert galactose to glucose 1-phosphate during galactose utilization and convert UDP-glucose to UDP-galactose which is required for E. coli cell wall biosynthesis. It is possible that the P2 promoter of the Streptomyces galK operon also serves to supply epimerase and galactokinase in the absence of galactose or during secondary metabolism.

EXAMPLE 6

THE S. COELICOLOR GAL OPERON

(i) Summary

The restriction map of a fragment containing the S. coelicolor galK gene is identical to the restriction map of the S. lividans gal operon. (See, Figure 3).

S. coelicolor can grow on minimal media containing galactose as the sole carbon source.

Galactokinase expression in S. coelicolor is induced by the addition of galactose to the growth media.

A promoter analogous and most likely identical to P1 is responsible for galactose induction of the S. coelicolor gal operon.

(ii) Experimental

An approximately 14 kb partial Sau3A fragment containing the S. coelicolor galK gene was isolated by K. Kendall and J. Cullum at the University of Manchester Institute of Science and Technology, Manchester, UK (unpublished data; personal communication). They were able to localize the S. coelicolor galK gene within a 3 kb EcoRI fragment by complementation of a S. coelicolor galK mutant. The position of a number of restriction sites within the S. lividans gal operon are identical to those found within, upstream and downstream of the EcoRI fragment containing the S. coelicolor galK gene (Figure 3). Thus, it seems likely that the gene organization of the S. coelicolor gal operon is identical to the S. lividans gal operon.

Galactose induction of the S. coelicolor galK gene was examined by immunoblotting. S. coelicolor was grown in YM + 1% galactose or 1% glucose (Ymglu or Ymgal) for 20 hours at 28 C. Galactokinase expression was detected using rabbit antisera prepared against purified E. coli galactokinase. The protein detected was the approximate site of the E. coli and S. lividans galK gene product. Galactokinase expression is galactose induced since it was detected only when S. coelicolor was grown in Ym + galactose (Ymgal).

S1 nuclease protection studies were performed to determine if galactose induction of the S. coelicolor gal operon is directed by a promoter analogous to the S. lividans P1 promoter. RNA was isolated from S. coelicolor grown in Ym + 1% galactose or 1% glucose (Ymgal or Ymglu). The hybridization probe used for S1 analysis of this RNA was a 410 bp Sau3A fragment which contains the S. lividans P1 promoter, its transcription start site and the 5' end of the galT gene. The S1 protected fragment detected by this analysis co-migrated with the protected fragment detected when the probe was hybridized to RNA isolated from S. lividans grown in the presence of galactose. Thus, this result shows that galactose induction of the S. coelicolor gal operon is directed by a sequence indistinguishable from the S. lividans P1 promoter.

It should be noted that the following strains of *Streptomyces* have been observed to be able to grow on medium containing galactose as the only carbon source:

S. albus J1074 (obtained from Dr. Chater, John Innes Foundation, Norwich, England)
S. carzinostaticus - ATCC accession number 15944
S. carzinostaticus - ATCC accession number 15945
S. antifibrinolyticus - ATCC accession number 21869
S. antifibrinolyticus - ATCC accession number 21870
S. antifibrinolyticus - ATCC accession number 21871
S. longisporus - ATCC accession number 23931

The abbreviation "ATCC" stands for the American Type Culture Collection, Rockville, Maryland, U.S.A.

While the above descriptions and Examples fully describe the invention and the preferred embodiments thereof, it is understood that the invention is not limited to the particular disclosed embodiments. Thus, the invention includes all embodiments coming within the scope of the following claims.

Claims for the Contracting States :
BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. A recombinant DNA molecule comprising a *Streptomyces gal* operon or any regulatable and functional derivative thereof.

2. The molecule of Claim 1 wherein the operon is a *S. lividans*, *S. coelicolor*, *S. azuraeus*, *S. albus*, *S. carzinostaticus*, *S. antifibrinolyticus* or *S. longisporus gal* operon.

3. The molecule of Claim 2 wherein the operon is a *S. lividans gal* operon.

4. The molecule of Claim 3 which has the following coding sequence:

```
      -120         -110          -100           -90          -80          -70
        .            .             .             .            .           .
CTA CGC CTC CGC GTT CAG TAA TTG AAC ACT TTT GGT GAT GAA CTT TGT TTG ATT GTG

            -60          -50          -40           -30          -20
             .            .            .             .            .
ATG TGA CAG CCG CGT GGT GGC TTG TGA TGT GTT ATG TTT GAT TGT GTT GCA TGA TTG
                                                          galP1

  -10            1          10           20           30            40
   .             .           .            .            .             .
ACG GGC GTC CTG GTG ACT CAT GGG TGG GTG CAG AGG AGT GCG GCA GTG AAG AAG ACC
                Met Thr His Gly Trp Val Gln Arg Ser Ala Ala Val Lys Lys Thr
                galT
     50           60            70           80           90            100
      .            .             .            .            .             .
TCG ACC CGG CTG GCC GAC GGC CGT GAG CTG GTC TAC TAC GAC CTG CGC GAC GAC ACC
Ser Thr Arg Leu Ala Asp Gly Arg Glu Leu Val Tyr Tyr Asp Leu Arg Asp Asp Thr

         110          120            130          140          150
          .            .             .            .            .
GTG CGC GAC GCC GTG GAC CGC CGT CCG CTG GAG CGG ACC GTC ACC ACG TCC GAG GTG
Val Arg Asp Ala Val Asp Arg Arg Pro Leu Glu Arg Thr Val Thr Thr Ser Glu Val

160          170          180           190          200          210
 .            .            .             .            .            .
CGA CGC GAC CCG CTG CTC GGC GAC TCC GCG CCG TCG CGC CTC GCA CCG GCA GGG GCC
Arg Arg Asp Pro Leu Leu Gly Asp Ser Ala Pro Ser Arg Leu Ala Pro Ala Gly Ala

    220          230          240           250          260          270
     .            .            .             .            .            .
CAC CTA CCA TCC GCC GGC CGA CCA GTG CCC GCT GTG CCc GTC GGA CGG GGA ACG GCT
His Leu Pro Ser Ala Gly Arg Pro Val Pro Ala Val Pro Val Gly Arg Gly Thr Ala

        280          290          300           310          320         330
         .            .            .             .            .           .
GAG CGA GAT CCG GCC TAT GAC GTG GTG GTC TTC GAG AAT CGC TTT CCC TCG CTG GCC
Glu Arg Asp Pro Ala Tyr Asp Val Val Val Phe Glu Asn Arg Phe Pro Ser Leu Ala
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            340         350          360            370         380
             .           .            .              .           .
GGT GAC TCC GGG CGC TGC GAG GTC GTC TGC TTC ACC TCC GAC CAC GAC GCC TCC TTC
Gly Asp Ser Gly Arg Cys Glu Val Val Cys Phe Thr Ser Asp His Asp Ala Ser Phe

390            400          410          420          430           440
 .              .            .            .            .             .
GCC GAC CTG AGC GAG GAG CAG GCC CGG CTG GTC GTC GAC GCC TGG ACG GAC CGC ACC
Ala Asp Leu Ser Glu Glu Gln Ala Arg Leu Val Val Asp Ala Trp Thr Asp Arg Thr

     450           460          470          480            490          500
      .             .            .            .              .            .
TCC GAG CTG TCC CAT CTG CCC TCC GTT GAA CAG GTG TTC TGC TTC GAG AAC CGG GGC
Ser Glu Leu Ser His Leu Pro Ser Val Glu Gln Val Phe Cys Phe Glu Asn Arg Gly

         510           520          530          540            550
          .             .            .            .              .
GCC GAG ATC GGG GTG ACG CTG GGT CAC CCG CAC GGC CAG ATC TAC GCC TAC CCG TTC
Ala Glu Ile Gly Val Thr Leu Gly His Pro His Gly Gln Ile Tyr Ala Tyr Pro Phe

560          570          580          590           600          610
 .            .            .            .             .            .
ACC ACC CCC CGC ACC GCC CTG ATG CTC CGT TCA CTC GCC GCC CAC AAG GAC GCG ACG
Thr Thr Pro Arg Thr Ala Leu Met Leu Arg Ser Leu Ala Ala His Lys Asp Ala Thr

   620          630           640          650          660           670
    .            .             .            .            .             .
GGC GGG GGG AAC CTC TTC GAC TCC GTG CTG GAG GAG GAG CTG GCC GGT GAG CGG GTC
Gly Gly Gly Asn Leu Phe Asp Ser Val Leu Glu Glu Glu Leu Ala Gly Glu Arg Val

        680          690          700           710          720
         .            .            .             .            .
GTC CTG GAG GGT GAG CAC TGG GCC GCC TTC GTC GCG TAC GGC GCG CAC TGG CCG TAC
Val Leu Glu Gly Glu His Trp Ala Ala Phe Val Ala Tyr Gly Ala His Trp Pro Tyr

730          740          750          760           770          780
 .            .            .            .             .            .
GAG GTG CAC CTC TAC CCG AAG CGG CGG GTG CCC GAT CTG CTC GGG CTC GAC GAG GCG
Glu Val His Leu Tyr Pro Lys Arg Arg Val Pro Asp Leu Leu Gly Leu Asp Glu Ala

   790          800           810          820          830           840
    .            .             .            .            .             .
GCT CGC ACA GAA TTC CCC AAG GTC TAC CTG GAG CTG CTG AGG CGT TTC GAC CGG ATC
Ala Arg Thr Glu Phe Pro Lys Val Tyr Leu Glu Leu Leu Arg Arg Phe Asp Arg Ile
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       850          860          870          880          890          900
        .            .            .            .            .            .
TTC GGC GAG GGC GAG CCC CCG ACC CCC TAC ATC GCG GCC TGG CAC CAG GCG CCG TTC
Phe Gly Glu Gly Glu Pro Pro Thr Pro Tyr Ile Ala Ala Trp His Gln Ala Pro Phe

           910          920          930          940          950
            .            .            .            .            .
GGC CAG CTG GAG TTC GAG GGT GTC ACG CGC GAC GAC TTC GCG CTC CAC CTG GAA CTT
Gly Gln Leu Glu Phe Glu Gly Val Thr Arg Asp Asp Phe Ala Leu His Leu Glu Leu

  960          970          980          990          1000         1010
   .            .            .            .            .            .
TTC ACT TCC GCC GTA CGT CCG GCA AGC TGA AGT TCC TCG CGG GCT CCG AAT CCG GCA
Phe Thr Ser Ala Val Arg Pro Ala Ser ---          ^galP2

      1020       1030       1040       1050       1060       1070
       .          .          .          .          .          .
  TGAACG TGTTCATCAA CGACGTACCC CCGGAGCGCG CGGCCGAGCG ACTGCGAGAG GTAGCGAC


1080         1090          1100         1110         1120         1130
 .            .             .            .            .            .
TTC ATG AGC GGG AAG TAC CTG GTG ACA GGT GGT GCC GGA TAC GTC GGC AGC GTC GTC
    Met Ser Gly Lys Tyr Leu Val Thr Gly Gly Ala Gly Tyr Val Gly Ser Val Val
    galE
    1140         1150          1160         1170         1180         1190
     .            .             .            .            .            .
GCC CAG CAC TTG GTG GAG GCG GGC AAC GAG GTC GTG GTG CTG CAC AAT CTG TCG ACC
Ala Gln His Leu Val Glu Ala Gly Asn Glu Val Val Val Leu His Asn Leu Ser Thr

        1200         1210          1220         1230         1240
         .            .             .            .            .
GGC TTC CGT GAG GTG TGC CGG CGC GTG CCT CGT TCG TCG AGG CGA CAT CCG GGA CGC
Gly Phe Arg Glu Val Cys Arg Arg Val Pro Arg Ser Ser Arg Arg His Pro Gly Arg

1250        1260         1270          1280         1290         1300
 .           .            .             .            .            .
CGC CAA GTG CGT GGA CGG CTC TCG TTC GAC GGC GTG CTG CAC TTC GCC GCC TTC TCC
Arg Gln Val Arg Gly Arg Leu Ser Phe Asp Gly Val Leu His Phe Ala Ala Phe Ser

    1310        1320         1330          1340         1350         1360
     .           .            .             .            .            .
CAG GTC GGC GAG TCG GTC GTG AAG CCC GAG AAG TAC TGG GAC AAC AAC GTC GGT GGC
Gln Val Gly Glu Ser Val Val Lys Pro Glu Lys Tyr Trp Asp Asn Asn Val Gly Gly
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      1370         1380          1390         1400         1410          1420
        .            .             .            .            .             .
ACC ATG GCG CTG CTC GAG GCC ATG CGC GGC GCC GGT GTG CGG CGC CTC GTC TTC TCC
Thr Met Ala Leu Leu Glu Ala Met Arg Gly Ala Gly Val Arg Arg Leu Val Phe Ser

          1430         1440         1450          1460         1470
            .            .            .             .            .
TCC ACG GCC GCC ACG TAC GGC GAG CCC GAG CAG GTT CCC ATC GTC GAG TCC GCG CCG
Ser Thr Ala Ala Thr Tyr Gly Glu Pro Glu Gln Val Pro Ile Val Glu Ser Ala Pro

1480          1490         1500         1510          1520         1530
  .             .            .            .             .            .
ACG AGG CCC ACC AAT CCG TAC GGC GCC TCG AAG CTC GCC GTC GAC CAC ATG ATC ACC
Thr Arg Pro Thr Asn Pro Tyr Gly Ala Ser Lys Leu Ala Val Asp His Met Ile Thr

    1540          1550         1560         1570          1580         1590
      .             .            .            .             .            .
GGC GAG GCG GCG GCC CAC GGG CTG GGC GCG GTC TCC GTG CCG TAC TTC AAC GTC GCG
Gly Glu Ala Ala Ala His Gly Leu Gly Ala Val Ser Val Pro Tyr Phe Asn Val Ala

        1600          1610         1620         1630          1640
          .             .            .            .             .
GGC GCG TAC GGG GAG TAC GGC GAG CGC CAC GAC CCC GAG TCG CAT CTG ATT CCG CTG
Gly Ala Tyr Gly Glu Tyr Gly Glu Arg His Asp Pro Glu Ser His Leu Ile Pro Leu

1650        1660          1670         1680         1690          1700
 .            .             .            .            .             .
GTC CTT CAA GTG GCG CAG GGC AGG CGG GAG GCC ATC TCC GTC TAC GGC GAC GAC TAC
Val Leu Gln Val Ala Gln Gly Arg Arg Glu Ala Ile Ser Val Tyr Gly Asp Asp Tyr

   1710         1720          1730         1740         1750          1760
     .            .             .            .            .             .
CCG ACC CCG GAC CGA CCT GTG TGC GCG ACT ACA TCC ACG TCG CCG ACC TGG CCG AGG
Pro Thr Pro Asp Arg Pro Val Cys Ala Thr Thr Ser Thr Ser Pro Thr Trp Pro Arg

       1770         1780          1790         1800         1810
         .            .             .            .            .
CCC ACC TGC TGG CCG TGC GCC GCC GCC CCG GGC GAG CAC CTC ATC TGC AAC CTG GGC
Pro Thr Cys Trp Pro Cys Ala Ala Ala Pro Gly Glu His Leu Ile Cys Asn Leu Gly

1820       1830         1840          1850         1860         1870
.            .            .             .            .            .
AAC GGC AAC GGC TTC TCC GTC CGC GAG GTC GTC GAG ACC GTG CGG CGG GTG ACG GGC
Asn Gly Asn Gly Phe Ser Val Arg Glu Val Val Glu Thr Val Arg Arg Val Thr Gly
```

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  1880         1890         1900          1910         1920         1930
    .            .            .             .            .            .
CAT CCC ATC CCC GAG ATC ATG GCC CCG CGC CGC GGG CGC GAC CCG GCG GTC CTG GTC
His Pro Ile Pro Glu Ile Met Ala Pro Arg Arg Gly Arg Asp Pro Ala Val Leu Val

       1940         1950         1960          1970         1980         1990
         .            .            .             .            .            .
GCG TCG GCC GGC ACC GCC CGC GAG AAG CTG GGC TGG AAC CCG TCC CGC GCG GAC CTC
Ala Ser Ala Gly Thr Ala Arg Glu Lys Leu Gly Trp Asn Pro Ser Arg Ala Asp Leu

           2000         2010         2020          2030         2040
             .            .            .             .            .
GCC ATC GTG TCG GAC GCG TGG GAG TTG CCG CAG CGC CGC GCG GGC CAG TAG  TA
Ala Ile Val Ser Asp Ala Trp Glu Leu Pro Gln Arg Arg Ala Gly Gln ---

       2050         2060         2070          2080         2090         2100
         .            .            .             .            .            .
ACC GCA GTT ACC GGA AAG GCG AGC GGT CAG GGC ATG GGC GAG GCT GTC GGG GAA CCG
                                            Met Gly Glu Ala Val Gly Glu Pro
                                            galK
           2110         2120         2130          2140         2150
             .            .            .             .            .
TCG GCG AGC GGT TCC GGG AGC TGT ACG GGC CGG AGC CGG AGG GGG TGT GGG CGC CGA
Ser Ala Ser Gly Ser Gly Ser Cys Thr Gly Arg Ser Arg Arg Gly Cys Gly Arg Arg

 2160         2170         2180          2190         2200         2210
   .            .            .             .            .            .
GCG GGC CGG GAG AAC CTC ATC GGG GAG CAC ACC GAC TAC AAC GAC GGC TTC GTC ATG
Ala Gly Arg Glu Asn Leu Ile Gly Glu His Thr Asp Tyr Asn Asp Gly Phe Val Met

     2220         2230         2240          2250         2260         2270
       .            .            .             .            .            .
CCT TCG CCC TGC CGC ACC AGG TCG CGC CCG TCT CCC GGC GCG AAC GAC GGC ATC CTC
Pro Ser Pro Cys Arg Thr Arg Ser Arg Pro Ser Pro Gly Ala Asn Asp Gly Ile Leu

         2280         2290         2300          2310         2320
           .            .            .             .            .
CGC CTG CAC TCG GCC GAC GTC GAC GCC GAC CCG GTC GAG CTG CGC GTC GCC GAC CTG
Arg Leu His Ser Ala Asp Val Asp Ala Asp Pro Val Glu Leu Arg Val Ala Asp Leu

2330        2340         2350          2360         2370         2380
 .            .            .             .            .            .
GCC CCC GCG TCG GAC AAG TCC TGG ACG GCG TAC CCC TCG GGC GTC CTG TGG GCG CTG
Ala Pro Ala Ser Asp Lys Ser Trp Thr Ala Tyr Pro Ser Gly Val Leu Trp Ala Leu
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    2390       2400         2410      2420      2430      2440
CGC GAG GCC GGA CAC GAG CTG ACC GGC GCC GAC GTC CAC CTG GCC TCG ACC GTC CCG
Arg Glu Ala Gly His Glu Leu Thr Gly Ala Asp Val His Leu Ala Ser Thr Val Pro

        2450      2460        2470      2480      2490
TCC GGG GCC GGG CTC TCC TCC TCC GCG GCC CTG GAG GTC CGT CCC CTG GCG ATG AAC
Ser Gly Ala Gly Leu Ser Ser Ser Ala Ala Leu Glu Val Arg Pro Leu Ala Met Asn

2500        2510       2520        2530        2540        2550
GAC CTG TAC GCC CTC GCG CTG CGC GGC TGG CAG CTG GCC CGG CTG TGC CAG CGC GCG
Asp Leu Tyr Ala Leu Ala Leu Arg Gly Trp Gln Leu Ala Arg Leu Cys Gln Arg Ala

    2560        2570        2580        2590        2600        2610
GAG AAC GTC TAC GTC GGC GCC CCC GTC GGC ATC ATG GAC CAG ACC GCG TCC GCC TGC
Glu Asn Val Tyr Val Gly Ala Pro Val Gly Ile Met Asp Gln Thr Ala Ser Ala Cys

        2620        2630        2640        2650        2660        2670
TGC GAG GCG GGC ACG CCC TCT TCC TCG ACA CCC GCG ACC TCT CCC AGC GGC AGA TCC
Cys Glu Ala Gly Thr Pro Ser Ser Ser Thr Pro Ala Thr Ser Pro Ser Gly Arg Ser

            2680        2690        2700        2710        2720
CCT TCG ACC TCG CCG CCG AGG GGA TGC GCC TGC TGG TCG TCG ACA CCC GGG TCA AGC
Pro Ser Thr Ser Pro Pro Arg Gly Cys Ala Cys Trp Ser Ser Thr Pro Gly Ser Ser

2730        2740        2750        2760        2770        2780
ACT CCC ACA GCG AGG GCG AGT ACG GCA AGC GCC GCG CGG GCT GCG AGA AGG GCG CCG
Thr Pro Thr Ala Arg Ala Ser Thr Ala Ser Ala Ala Arg Ala Ala Arg Arg Ala Pro

    2790        2800        2810        2820        2830        2840
CGC TGC TGG GCG TCG ACG CGC TGC GAC GTG CCG TAC GCC GAC CTG GAC GCG GCG CTG
Arg Cys Trp Ala Ser Thr Arg Cys Asp Val Pro Tyr Ala Asp Leu Asp Ala Ala Leu

        2850        2860        2870        2880        2890
GAG CGG CTG GGC GAC GAG GAG GAG GTG CGC CGC CTG GTC CGG CAC GTG GTC ACC GAG
Glu Arg Leu Gly Asp Glu Glu Glu Val Arg Arg Leu Val Arg His Val Val Thr Glu
```

```
2900        2910          2920         2930         2940          2950
 .            .             .            .            .             .
GAC GAG CGC GTC GAA CGG GTG GTC GCG CTG CTG GAG TCG GCG ACA CCC GGC GCA TCG
Asp Glu Arg Val Glu Arg Val Val Ala Leu Leu Glu Ser Ala Thr Pro Gly Ala Ser

   2960         2970          2980         2990         3000          3010
     .            .             .            .            .             .
GCG CCC TCC TGG TCG AGG GCC ACG CCT GCT GCG CGA CGA CTT CCG CAT CTC CTG CCC
Ala Pro Ser Trp Ser Arg Ala Thr Pro Ala Ala Arg Arg Leu Pro His Leu Leu Pro

        3020        3030           3040        3050         3060
          .           .              .           .            .
CGA GCT GGA CCT GGT CGT CGA CAC GGC CCT GGC CTC CGC GGC CCT CGG CGC CGG ATG
Arg Ala Gly Pro Gly Arg Arg His Gly Pro Gly Leu Arg Gly Pro Arg Arg Arg Met

3070        3080        3090           3100        3110         3120
.             .           .              .           .            .
ACC GGC GGC GGC TTC GGC GGC TCG GCG ATC GTC CTG GTG GAG GCC GCC GCG GTG GAC
Thr Gly Gly Gly Phe Gly Gly Ser Ala Ile Val Leu Val Glu Ala Ala Ala Val Asp

   3130         3140        3150          3160         3170         3180
     .            .           .             .            .            .
GCC GTC ACC AAG GCG GTC GAG GAC GCC TTC GCC GCG GCC GGC CTC AAG CGT CCG CGC
Ala Val Thr Lys Ala Val Glu Asp Ala Phe Ala Ala Ala Gly Leu Lys Arg Pro Arg

      3190         3200         3210          3220         3230         3240
        .            .            .             .            .            .
GTG TTC GAG GCG GTG CCT CGG CGG GGC GCG GCG CCT GGT CTG ACG GTC AGC CGA GCC
Val Phe Glu Ala Val Pro Arg Arg Gly Ala Ala Pro Gly Leu Thr Val Ser Arg Ala

           3250        3260         3270          3280         3290
             .           .            .             .            .
GCT TCA CCA GCG TGT ACT CCC TGA TCC CCG GCG GGT AGT CGG GGA TCA CGC ACA TGA
Ala Ser Pro Ala Cys Thr Pro ---

3300
  .
GCT GCT AGC CGC
```

5. The molecule of Claim 1 which further comprises a foreign functional DNA sequence operatively linked to such operon.

6. A transformed host microorganism or cell comprising the molecule of Claim 5.

7. A method of preparing a transformed host microorganism or cell comprising the molecule of Claim 5 which comprises transforming an appropriate host microorganism or cell with such molecule.

8. A recombinant DNA vector comprising the molecule of Claim 5, and, optionally, additionally comprising a replicon.

9. A transformed host microorganism or cell comprising the recombinant DNA vector of Claim 8.

10. A method of preparing a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 8 which comprises transforming an appropriate host microorganism or cell with such vector.

11. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 8 under suitable conditions such that the functional DNA sequence is expressed.

12. A method of regulating the expression of a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell which contains the recombinant DNA vector of Claim 8 under appropriate conditions such that expression of the sequence is regulatable.

13. A recombinant DNA molecule comprising a <u>Streptomyces</u> <u>gal</u> operon P2 promoter expression unit or any functional derivative thereof.

14. The molecule of Claim 13 wherein the expression unit is a <u>S</u>. <u>lividans</u>, <u>S.</u> <u>coelicolor</u>, <u>S</u>. <u>azuraeus</u>, <u>S</u>. <u>albus</u>, <u>S</u>. <u>carzinostaticus</u>, <u>S</u>. <u>antifibrinolyticus</u> or <u>S</u>. <u>longisporus</u> <u>gal</u> operon P2 promoter expression unit.

15. The molecule of Claim 14 which is a *S. lividans gal* operon P2 promoter expression unit.

16. The molecule of Claim 13 which further comprises a foreign functional DNA sequence operatively linked to such expression unit.

17. A transformed host microorganism or cell comprising a recombinant DNA molecule wherein such molecule comprises the molecule of Claim 16.

18. A method of preparing a transformed host microorganism comprising the molecule of Claim 16 which comprises transforming an appropriate host microorganism or cell with such molecule.

19. A recombinant DNA vector comprising the molecule of Claim 16, and, optionally, additionally comprising a replicon.

20. A transformed host microorganism or cell comprising the recombinant DNA vector of Claim 19.

21. A method of preparing a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 19 which comprises transforming an appropriate host microorganism with such vector.

22. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 19 under suitable conditions such that the functional DNA sequence is expressed.

23. A recombinant DNA molecule comprising a *Streptomyces gal* operon P1 promoter regulated region or any regulatable and functional derivative thereof.

24. The molecule of Claim 23 wherein the region is a *S. lividans*, *S. coelicolor*, *S. azuraeus*, *S. albus*, *S. carzinostaticus*, *S. antifibrinolyticus* or *S. longisporus gal* operon P1 promoter regulated region.

25. The molecule of Claim 24 wherein the region is a *S. lividans gal* operon P1 promoter regulated region.

26. The molecule of Claim 23 which further comprises a foreign functional DNA sequence operatively linked to such regulated region.

27. A transformed host microorganism or cell comprising the molecule of Claim 26.

28. A method of preparing a transformed host microorganism or cell comprising the molecule of Claim 26 which comprises transforming an appropriate host microoganism or cell with such molecule.

29. A recombinant DNA vector comprising the molecule Claim 26, and, optionally, additionally comprising a replicon.

30. A transformed host microorganism or cell comprising a recombinant DNA vector of Claim 29.

31. A method of preparing a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 29 which comprises transforming an appropriate host microorganism or cell with such vector.

32. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 29 under suitable conditions such that the functional DNA sequence is expressed.

33. A method of regulating the expression of a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell which contains the recombinant DNA vector of Claim 29 under appropriate conditions such that expression of the sequence is regulatable.

34. A recombinant DNA molecule comprising a Streptomyces gal operon P2 promoter or any functional derivative thereof.

35. The molecule of Claim 34 wherein the promoter is a S. lividans, S. coelicolor, S. azuraeus, S. albus, S. carzinostaticus, S. antifibrinolyticus or S. longisporus gal operon P2 promoter.

36. The molecule of Claim 35 wherein the promoter is a *S. lividans gal* operon P2 promoter.

37. The molecule of Claim 34 which further comprises a foreign functional DNA sequence operatively linked to the P2 promoter.

38. A transformed host microorganism or cell comprising the molecule of Claim 37.

39. A method of preparing a transformed host microorganism or cell comprising the molecule of Claim 37 which comprises transforming an appropriate host microorganism or cell with such molecule.

40. A recombinant DNA vector comprising the molecule of Claim 37 and, optionally, additionally comprising a replicon.

41. A transformed host microorganism or cell comprising the recombinant DNA vector of Claim 40.

42. A method of preparing a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 40 which comprises transforming an appropriate host microorganism with such vector.

43. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 40 under suitable conditions such that the functional DNA sequence is expressed.

44. A recombinant DNA molecule comprising a *Streptomyces gal* operon P1 promoter or any regulatable and functional derivative thereof.

45. The molecule of Claim 44 wherein the promoter is a *S. lividans*, *S. coelicolor*, *S. azuraeus*, *S. albus*, *S. carzinostaticus*, *S. antifibrinolyticus* or *S. longisporus gal* operon P1 promoter.

46. The molecule of Claim 45 wherein the promoter is a *S. lividans gal* operon P1 promoter.

47. The molecule of Claim 44 which further comprises a foreign functional DNA sequence operatively linked to the P1 promoter.

48. A transformed host microorganism or cell comprising the molecule of Claim 47.

49. A method of preparing a transformed host microorganism or cell comprising molecule of Claim 47 which comprises transforming an appropriate host microorganism or cell with such molecule.

50. A recombinant DNA vector comprising the molecule of Claim 47, and, optionally, additionally comprising a replicon.

51. A transformed host microorganism or cell comprising the recombinant DNA vector of Claim 50.

52. A method of preparing a transformed host microorganism or cell comprising the recombinant DNA vector of claim 50 which comprises transforming an appropriate host microorganism with such vector.

53. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 50 under suitable conditions such that the functional DNA sequence is expressed.

54. A method of regulating the expression of a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell which contains the recombinant DNA vector of Claim 50 under appropriate conditions such that expression of the sequence is regulatable.

55. A recombinant DNA molecule comprising a <u>Streptomyces</u> <u>gal</u> operon <u>gal</u>E gene, or any functional derivative thereof.

56. The molecule of Claim 55 wherein the gene is a <u>S</u>. <u>lividans</u>, <u>S</u>. <u>coelicolor</u>, <u>S</u>. <u>azuraeus</u>, <u>S</u>. <u>albus</u>, <u>S</u>. <u>carzinostaticus</u>, <u>S</u>. <u>antifibrinolyticus</u> or <u>S</u>. <u>longisporus</u> <u>gal</u> operon <u>gal</u>E gene.

57. The molecule of Claim 56 wherein the gene is a <u>S</u>. <u>lividans</u> <u>gal</u> operon <u>gal</u>E gene.

58. The molecule of Claim 55 which further comprises a foreign functional DNA sequence operatively linked to the galE gene.

59. A transformed host microorganism or cell comprising the molecule of Claim 58.

60. A method of preparing a transformed host microoganism or cell comprising the molecule of Claim 58 which comprises transforming an appropriate host microorganism or cell with such molecule.

61. A recombinant DNA molecule comprising a Streptomyces gal operon galT gene, or any functional derivative thereof.

62. The molecule of Claim 61 wherein the gene is a S. lividans, S. coelicolor, S. azuraeus or S. albus, S. carzinostaticus, S. antifibrinolyticus and S. longisporus gal operon galT gene.

63. The molecule of Claim 62 wherein the gene is a S. lividans gal operon galT gene.

64. The molecule of Claim 61 which further comprises a foreign functional DNA sequence operatively linked to the galT gene.

65. A transformed host microorganism or cell comprising the molecule of Claim 64.

66. A method of preparing a transformed host microorganism or cell comprising the molecule of Claim 64 which comprises transforming an appropriate host microorganism or cell with such molecule.

67. A recombinant DNA molecule comprising a Streptomyces lividans gal operon galK gene, or any functional derivative thereof.

68. The molecule of Claim 67 wherein the gene is a S. lividans, S. coelicolor, S. azuraeus, S. albus, S. carzinostaticus, S. antifibrinolyticus or S. longisporus gal operon galK gene.

69. The molecule of Claim 68 wherein is a S. lividans gal operon galK gene.

70. The molecule of Claim 67 which further comprises a foreign functional DNA sequence operatively linked to the *gal*K gene.

71. A transformed host microorganism or cell comprising the molecule of Claim 70.

72. A method of preparing a transformed host microorganism or cell comprising the molecule of Claim 70 which comprises transforming an appropriate host microorganism or cell with such molecule.

73. A method of enabling a nongalactose utilizing host microorganism or cell to utilize galactose which comprises transforming such host with a recombinant DNA vector or molecule comprising a *Streptomyces gal* operon, or any portion of the *Streptomyces gal* operon which is adequate to enable such transformed host to utilize galactose, or any functional derivative thereof.

74. A transformed host prepared by the method of Claim 73.

Claims for the Contracting States : AT, ES, GR

1. A method of preparing a transformed host microorganism or cell comprising the molecule which has the following sequence :

```
       -120        -110        -100            -90         -80         -70
        .           .           .               .           .           .
CTA CGC CTC CGC GTT CAG TAA TTG AAC ACT TTT GGT GAT GAA CTT TGT TTG ATT GTC

           -60         -50         -40             -30         -20
            .           .           .               .           .
ATG TGA CAG CGG GCT GGT GGG TTG TGA TGT GTT ATG TTT GAT TGT GTT GGA TGA TTG
                                                             galP1

-10             1           10          20          30              40
.               .           .           .           .               .
ACG GGC GTC CTG GTG ACT CAT GGG TGG GTG CAG AGG AGT GCG GCA GTG AAG AAG ACC
                Met Thr His Gly Trp Val Gln Arg Ser Ala Ala Val Lys Lys Thr
                galT
    50          60              70          80          90              100
    .           .               .           .           .               .
TCG ACC CGG CTG GCC GAC GGC CGT GAG CTG GTC TAC TAC GAC CTG CGC GAC GAC ACC
Ser Thr Arg Leu Ala Asp Gly Arg Glu Leu Val Tyr Tyr Asp Leu Arg Asp Asp Thr

        110         120             130         140         150
        .           .               .           .           .
GTG CGC GAC GCC GTG GAC CGC CGT CCG CTG GAG CGG ACC GTC ACC ACG TCC GAG GTG
Val Arg Asp Ala Val Asp Arg Arg Pro Leu Glu Arg Thr Val Thr Thr Ser Glu Val

160         170         180             190         200         210
.           .           .               .           .           .
CGA CGC GAC CCG CTG CTC GGC GAC TCC GCG CCG TCG CGC CTC GCA CCG GCA GGG GCG
Arg Arg Asp Pro Leu Leu Gly Asp Ser Ala Pro Ser Arg Leu Ala Pro Ala Gly Ala

    220         230         240             250         260         270
    .           .           .               .           .           .
CAC CTA CCA TCC GCC GGC CGA CCA GTG CCC GCT GTG CCc GTC GGA CGG GGA ACG GCT
His Leu Pro Ser Ala Gly Arg Pro Val Pro Ala Val Pro Val Gly Arg Gly Thr Ala

        280         290         300             310         320         330
        .           .           .               .           .           .
GAG CGA GAT CCG GCC TAT GAC GTG GTG GTC TTC GAG AAT CGC TTT CCC TCG CTG GCC
Glu Arg Asp Pro Ala Tyr Asp Val Val Val Phe Glu Asn Arg Phe Pro Ser Leu Ala
```

340 350 360 370 380
GCT GAC TCC GGG CGC TGC GAG GTC GTC TGC TTC ACC TCC GAC CAC GAC GCC TCC TTC
Gly Asp Ser Gly Arg Cys Glu Val Val Cys Phe Thr Ser Asp His Asp Ala Ser Phe

390 400 410 420 430 440
GCC GAC CTG AGC GAG GAG CAG GCC CGG CTG GTC GTC GAC GCC TGG ACG GAC CGC ACC
Ala Asp Leu Ser Glu Glu Gln Ala Arg Leu Val Val Asp Ala Trp Thr Asp Arg Thr

450 460 470 480 490 500
TCC GAG CTG TCC CAT CTG CCC TCC GTT GAA CAG GTG TTC TGC TTC GAG AAC CGC GGC
Ser Glu Leu Ser His Leu Pro Ser Val Glu Gln Val Phe Cys Phe Glu Asn Arg Gly

510 520 530 540 550
GCC GAG ATC GGG GTG ACG CTG GGT CAC CCG CAC GGG CAG ATC TAC GCC TAC CCG TTC
Ala Glu Ile Gly Val Thr Leu Gly His Pro His Gly Gln Ile Tyr Ala Tyr Pro Phe

560 570 580 590 600 610
ACC ACC CCC CGC ACC GCC CTG ATG CTC CGT TCA CTC GCC GCC CAC AAG GAC GCG ACG
Thr Thr Pro Arg Thr Ala Leu Met Leu Arg Ser Leu Ala Ala His Lys Asp Ala Thr

620 630 640 650 660 670
GGC GGC GGG AAC CTG TTC GAC TCC GTG CTG GAG GAG GAG CTG GCC GGT GAG CGC GTC
Gly Gly Gly Asn Leu Phe Asp Ser Val Leu Glu Glu Glu Leu Ala Gly Glu Arg Val

680 690 700 710 720
GTC CTG GAG GGT GAG CAC TGG GCC GCC TTC GTC GCG TAC GGC GCG CAC TGG CCG TAC
Val Leu Glu Gly Glu His Trp Ala Ala Phe Val Ala Tyr Gly Ala His Trp Pro Tyr

730 740 750 760 770 780
GAG GTG CAC CTC TAC CCG AAG CGC CGG GTG CCC GAT CTG CTC GGG CTC GAC GAG GCG
Glu Val His Leu Tyr Pro Lys Arg Arg Val Pro Asp Leu Leu Gly Leu Asp Glu Ala

790 800 810 820 830 840
GCT CGC ACA GAA TTC CCC AAG GTC TAC CTG GAG CTG CTG AGG CGT TTC GAC CGG ATC
Ala Arg Thr Glu Phe Pro Lys Val Tyr Leu Glu Leu Leu Arg Arg Phe Asp Arg Ile

```
        850         860         870             880         890         900
         .           .           .               .           .           .
TTC GCC GAG GCC GAG CCC CCG ACC CCC TAC ATC GCG GCC TGG CAC CAG GCG CCG TTC
Phe Gly Glu Gly Glu Pro Pro Thr Pro Tyr Ile Ala Ala Trp His Gln Ala Pro Phe

            910         920         930             940         950
             .           .           .               .           .
GGC CAG CTG GAG TTC GAG GGT GTG ACG CGC GAC GAC TTC GCG CTC CAC CTG GAA CTT
Gly Gln Leu Glu Phe Glu Gly Val Thr Arg Asp Asp Phe Ala Leu His Leu Glu Leu

960             970         980         990         1000            1010
 .               .           .           .           .               .
TTC ACT TCC GCC GTA CGT CCG GCA AGC TGA AGT TCC TCG CGG GCT CCG AAT CCG GCA
Phe Thr Ser Ala Val Arg Pro Ala Ser ---            ^galP2

 1020       1030       1040       1050       1060       1070
   .          .          .          .          .          .
TGAACC TGTTCATCAA CGACGTACCC CCGGAGCGCG CGGCCGAGCG ACTGCGAGAG GTAGCGAG

1080        1090            1100        1110        1120            1130
 .           .               .           .           .               .
TTC ATG AGC GGG AAG TAC CTG GTG ACA GGT GGT GCC GGA TAC GTC GGC AGC GTC GTC
    Met Ser Gly Lys Tyr Leu Val Thr Gly Gly Ala Gly Tyr Val Gly Ser Val Val
    galE
    1140        1150        1160            1170        1180            1190
     .           .           .               .           .               .
GCC CAG CAC TTG GTG GAG GCG GGG AAC GAG GTC GTG GTG CTG CAC AAT CTG TCG ACC
Ala Gln His Leu Val Glu Ala Gly Asn Glu Val Val Val Leu His Asn Leu Ser Thr

    1200            1210        1220            1230        1240
     .               .           .               .           .
GGC TTC CGT GAG GTG TGC CGG CGG GTG CCT CGT TCG TCG AGG CGA CAT CCG GGA CGC
Gly Phe Arg Glu Val Cys Arg Arg Val Pro Arg Ser Ser Arg Arg His Pro Gly Arg

1250        1260        1270            1280        1290        1300
 .           .           .               .           .           .
CGC CAA GTG CGT GGA CGG CTC TCG TTC GAC GGC GTG CTG CAC TTC GCC GCC TTC TCC
Arg Gln Val Arg Gly Arg Leu Ser Phe Asp Gly Val Leu His Phe Ala Ala Phe Ser

    1310        1320        1330            1340        1350        1360
     .           .           .               .           .           .
CAG GTC GGC GAG TCG GTC GTG AAG CCC GAG AAG TAC TGG GAC AAC AAC GTC GGT GGC
Gln Val Gly Glu Ser Val Val Lys Pro Glu Lys Tyr Trp Asp Asn Asn Val Gly Gly
```

1370 1380 1390 1400 1410 1420
ACC ATG GCG CTG CTG GAG GCC ATG CGG GGC GCG GGT GTG CGG CGG CTC GTC TTC TCC
Thr Met Ala Leu Leu Glu Ala Met Arg Gly Ala Gly Val Arg Arg Leu Val Phe Ser

1430 1440 1450 1460 1470
TCC ACG GCC GCC ACG TAC GGC GAC CCC GAG CAG GTT CCC ATC GTC GAG TCC GCG CCG
Ser Thr Ala Ala Thr Tyr Gly Glu Pro Glu Gln Val Pro Ile Val Glu Ser Ala Pro

1480 1490 1500 1510 1520 1530
ACG AGG CCC ACC AAT CCG TAC GGC GCC TCG AAG CTC GCC GTC GAC CAC ATG ATC ACC
Thr Arg Pro Thr Asn Pro Tyr Gly Ala Ser Lys Leu Ala Val Asp His Met Ile Thr

1540 1550 1560 1570 1580 1590
GGC GAG GCG GCG GCC CAC GGG CTG GGC GCG GTC TCC GTG CCG TAC TTC AAC GTC GCG
Gly Glu Ala Ala Ala His Gly Leu Gly Ala Val Ser Val Pro Tyr Phe Asn Val Ala

1600 1610 1620 1630 1640
GGC GCG TAC GGG GAG TAC GGC GAG CGC CAC GAC CCC GAG TCG CAT CTG ATT CCG CTG
Gly Ala Tyr Gly Glu Tyr Gly Glu Arg His Asp Pro Glu Ser His Leu Ile Pro Leu

1650 1660 1670 1680 1690 1700
GTC CTT CAA GTG GCG CAG GGC AGG CGG GAG GCC ATC TCC GTC TAC GGC GAC GAC TAC
Val Leu Gln Val Ala Gln Gly Arg Arg Glu Ala Ile Ser Val Tyr Gly Asp Asp Tyr

1710 1720 1730 1740 1750 1760
CCG ACC CCG GAC CGA CCT GTG TGC GCG ACT ACA TCC ACG TCG CCG ACC TGG CCG AGG
Pro Thr Pro Asp Arg Pro Val Cys Ala Thr Thr Ser Thr Ser Pro Thr Trp Pro Arg

1770 1780 1790 1800 1810
CCC ACC TGC TGG CCG TGC GCC GCC GCC CCG GGC GAG CAC CTC ATC TGC AAC CTG GGC
Pro Thr Cys Trp Pro Cys Ala Ala Ala Pro Gly Glu His Leu Ile Cys Asn Leu Gly

1820 1830 1840 1850 1860 1870
AAC GGC AAC GGC TTC TCC GTC CGC GAG GTC GTC GAG ACC GTG CGG CGG GTG ACG GGC
Asn Gly Asn Gly Phe Ser Val Arg Glu Val Val Glu Thr Val Arg Arg Val Thr Gly

```
    1880        1890        1900            1910        1920        1930
     .           .           .               .           .           .
CAT CCG ATC CCC GAG ATC ATG GCC CCC CGC CGC GGC CGC GAC CCG GCG GTC CTG GTC
His Pro Ile Pro Glu Ile Met Ala Pro Arg Arg Gly Arg Asp Pro Ala Val Leu Val

        1940        1950        1960            1970        1980        1990
         .           .           .               .           .           .
GCG TCG GCC GGC ACC GCC CGC GAG AAG CTG GGC TGG AAC CCG TCC CGC GCG GAC CTC
Ala Ser Ala Gly Thr Ala Arg Glu Lys Leu Gly Trp Asn Pro Ser Arg Ala Asp Leu

            2000        2010        2020            2030        2040
             .           .           .               .           .
GCC ATC GTG TCG GAC GCG TGG GAG TTG CCG CAG CGG CGC GCG GGC CAG TAG  TA
Ala Ile Val Ser Asp Ala Trp Glu Leu Pro Gln Arg Arg Ala Gly Gln ---

        2050        2060        2070            2080        2090        2100
         .           .           .               .           .           .
ACC GCA GTT ACC GGA AAG GCG AGG GGT CAG GGC ATG GGC GAG GCT GTC GGG GAA CCG
                                            Met Gly Glu Ala Val Gly Glu Pro
                                            galK
            2110        2120        2130            2140        2150
             .           .           .               .           .
TCG GCG AGC GGT TCC GGG AGC TGT ACG GGG CGG AGC CGG AGG GGG TGT GGG CGC CGA
Ser Ala Ser Gly Ser Gly Ser Cys Thr Gly Arg Ser Arg Arg Gly Cys Gly Arg Arg

2160            2170        2180        2190            2200        2210
 .               .           .           .               .           .
GCG GGC CGG GAG AAC CTC ATC GGG GAG CAC ACC GAC TAC AAC GAC GGC TTC GTC ATG
Ala Gly Arg Glu Asn Leu Ile Gly Glu His Thr Asp Tyr Asn Asp Gly Phe Val Met

    2220            2230        2240        2250            2260        2270
     .               .           .           .               .           .
CCT TCG CCC TGC CGC ACC AGG TCG CGG CCG TCT CCC GGC GCG AAC GAC GGC ATC CTG
Pro Ser Pro Cys Arg Thr Arg Ser Arg Pro Ser Pro Gly Ala Asn Asp Gly Ile Leu

        2280            2290        2300        2310            2320
         .               .           .           .               .
CGC CTG CAC TCG GCC GAC GTC GAC GCC GAC CCG GTC GAG CTG CGC GTC GCC GAC CTG
Arg Leu His Ser Ala Asp Val Asp Ala Asp Pro Val Glu Leu Arg Val Ala Asp Leu

2330        2340            2350        2360            2370        2380
 .           .               .           .               .           .
GCC CCC GCG TCG GAC AAG TCC TGG ACG GCG TAC CCC TCG GGC GTC CTG TGG GCG CTG
Ala Pro Ala Ser Asp Lys Ser Trp Thr Ala Tyr Pro Ser Gly Val Leu Trp Ala Leu
```

2390 2400 2410 2420 2430 2440

CGC GAG GCC GGA CAC GAG CTG ACC GGC GCC GAC GTC CAC CTC GCC TCG ACC GTC CCG

Arg Glu Ala Gly His Glu Leu Thr Gly Ala Asp Val His Leu Ala Ser Thr Val Pro

2450 2460 2470 2480 2490

TCC GGG GCC GGG CTC TCC TCC TCC GCG GCC CTG GAG GTC CGT CCC CTG GCG ATG AAC

Ser Gly Ala Gly Leu Ser Ser Ser Ala Ala Leu Glu Val Arg Pro Leu Ala Met Asn

2500 2510 2520 2530 2540 2550

GAC CTC TAC GCC CTC GCG CTG CGC GGC TGG CAG CTG GCC CGG CTG TGC CAG CGC GCG

Asp Leu Tyr Ala Leu Ala Leu Arg Gly Trp Gln Leu Ala Arg Leu Cys Gln Arg Ala

2560 2570 2580 2590 2600 2610

GAG AAC GTC TAC GTC GGC GCC CCC GTC GGC ATC ATG GAC CAG ACG GCG TCC GCC TGC

Glu Asn Val Tyr Val Gly Ala Pro Val Gly Ile Met Asp Gln Thr Ala Ser Ala Cys

2620 2630 2640 2650 2660 2670

TGC GAG GCG GGC ACG CCC TCT TCC TCG ACA CCC GCG ACC TCT CCC AGC GGC AGA TCC

Cys Glu Ala Gly Thr Pro Ser Ser Ser Thr Pro Ala Thr Ser Pro Ser Gly Arg Ser

2680 2690 2700 2710 2720

CCT TCG ACC TCG CCG CCG AGG GGA TGC GCC TGC TGG TCG TCG ACA CCC GGG TCA AGC

Pro Ser Thr Ser Pro Pro Arg Gly Cys Ala Cys Trp Ser Ser Thr Pro Gly Ser Ser

2730 2740 2750 2760 2770 2780

ACT CCC ACA GCC AGG GCG AGT ACG GCA AGC GCC GCG CGC GCT GCG AGA AGG GCG CCG

Thr Pro Thr Ala Arg Ala Ser Thr Ala Ser Ala Ala Arg Ala Ala Arg Arg Ala Pro

2790 2800 2810 2820 2830 2840

CGC TGC TGG GCG TCG ACG CGC TGC GAC GTG CCG TAC GCC GAC CTG GAC GCG GCG CTG

Arg Cys Trp Ala Ser Thr Arg Cys Asp Val Pro Tyr Ala Asp Leu Asp Ala Ala Leu

2850 2860 2870 2880 2890

GAG CGG CTG GGC GAC GAG GAG GAG GTG CGC CGC CTG GTC CGG CAC GTG GTG ACC GAG

Glu Arg Leu Gly Asp Glu Glu Glu Val Arg Arg Leu Val Arg His Val Val Thr Glu

```
2900        2910         2920          2930         2940         2950
.            .            .             .            .            .
GAC GAG CGC GTC GAA CGG GTG GTC GCG CTG CTG GAG TCG GCG ACA CCC GGC GCA TCG
Asp Glu Arg Val Glu Arg Val Val Ala Leu Leu Glu Ser Ala Thr Pro Gly Ala Ser

   2960         2970         2980          2990         3000         3010
    .            .            .             .            .            .
GCG CCC TCC TGG TCG AGG GCC ACG CCT GCT GCG CGA CGA CTT CCG CAT CTC CTG CCC
Ala Pro Ser Trp Ser Arg Ala Thr Pro Ala Ala Arg Arg Leu Pro His Leu Leu Pro

       3020         3030         3040          3050         3060
        .            .            .             .            .
CGA GCT GGA CCT GGT CGT CGA CAC GGC CCT GGC CTC CGC GGC CCT CGG CGC CGG ATG
Arg Ala Gly Pro Gly Arg Arg His Gly Pro Gly Leu Arg Gly Pro Arg Arg Arg Met

3070        3080         3090          3100         3110         3120
.            .            .             .            .            .
ACC GGC GGC GGC TTC GGC GGC TCG GCG ATC GTC CTG GTG GAG GCC GCC GCG GTG GAC
Thr Gly Gly Gly Phe Gly Gly Ser Ala Ile Val Leu Val Glu Ala Ala Ala Val Asp

   3130         3140         3150          3160         3170         3180
    .            .            .             .            .            .
GCC GTC ACC AAG GCG GTC GAG GAC GCC TTC GCC GCG GCG GGC CTC AAG CGT CCG CGG
Ala Val Thr Lys Ala Val Glu Asp Ala Phe Ala Ala Ala Gly Leu Lys Arg Pro Arg

       3190         3200         3210          3220         3230         3240
        .            .            .             .            .            .
GTG TTC GAG GCG GTG CCT CGG CGG GGC GCG GCG CCT GGT CTG ACG GTC AGC CGA GCC
Val Phe Glu Ala Val Pro Arg Arg Gly Ala Ala Pro Gly Leu Thr Val Ser Arg Ala

           3250         3260         3270          3280         3290
            .            .            .             .            .
GCT TCA CCA GCG TGT ACT CCG TGA TCC CCG GCG GGT AGT CGG GGA TCA CGC ACA TGA
Ala Ser Pro Ala Cys Thr Pro ---

 3300
  .
GCT GCT ACC CGC
```

which comprises transforming an appropriate host microorganism or cell with such molevule.

2. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA vector comprising the sequence of Claim 1 which comprises transforming an appropriate host microorganism or cell with such vector.

3. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 2 under suitable conditions such that the functional DNA sequence is expressed.

4. A method of regulating the expression of a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell which contains the recombinant DNA vector of Claim 2 under appropriate conditions such that expression of the sequence is regulatable.

5. A method of preparing a transformed host microorganism comprising a recombinant DNA molecule comprising a <u>Streptomyces</u> <u>gal</u> operon P2 promoter expression unit or any functional derivative thereof and a foreign functional DNA sequence operatively linked to such expression unit, which comprises transforming an appropriate host microorganism or cell with such molecule.

6. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 5 and, optionally, additionally comprising a replicon which comprises transforming an appropriate host microorganism with such vector.

7. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 5 and, optionally, additionally comprising a replicon, under suitable con-

ditions such that the functional DNA sequence is expressed.

8. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a <u>Streptomyces</u> <u>gal</u> operon P1 promoter regulated region or any regulatable and functional derivative thereof and a foreign functional DNA sequence operatively linked to such regulated region, which comprises transforming an appropriate host microorganism or cell with such molecule.

9. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 8 and, optionally, additionally comprising a replicon which comprises transforming an appropriate host microorganism or cell with such vector.

10. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 8 and, optionally, additionally comprising a replicon under suitable conditions such that the functional DNA sequence is expressed.

11. A method of regulating the expression of a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell which contains a recombinant DNA vector comprising the molecule of Claim 8 and, optionally, additionally comprising a replicon, under appropriate conditions such that expression of the sequence is regulatable.

12. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a <u>Streptomyces</u> <u>gal</u> operon P2 promoter or any functional derivative thereof and a foreign functional DNA sequence operatively linked to the P2

promoter, which comprises transforming an appropriate host microorganism or cell with such molecule.

13. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 12 and, optionally, additionally comprising a replicon, which comprises transforming an appropriate host microorganism with such vector.

14. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising a recombinant DNA molecule of Claim 12 and, optionally, additionally comprising a replicon, under suitable conditions such that the functional DNA sequence is expressed.

15. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a _Streptomyces_ _gal_ operon P1 promoter or any regulatable and functional DNA sequence operatively linked to the P1 promoter, which comprises transforming an appropriate host microorganism or cell with such molecule.

16. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 15 and, optionally, additionally comprising a replicon, which comprises transforming an appropriate host microorganism with such vector.

17. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 15 and, optionally, additionally comprising a replicon, under suitable conditions such that the functional DNA sequence is expressed.

18. A method of regulating the expression of a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell which contains a recombinant DNA vector comprising the molecule of Claim 15 and, optionally, additionally comprising a replicon, under appropriate conditions such that expression of the sequence is regulatable.

19. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a Streptomyces gal operon galE gene or any functional derivative thereof and a foreign functional DNA sequence operatively linked to the galE gene, which comprises transforming an appropriate host microorganism or cell with such molecule.

20. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a Streptomyces gal operon galT gene or any functional derivative thereof and a foreign functional DNA sequence operatively linked to the galT gene, which comprises transforming an appropriate host microorganism or cell with such molecule.

21. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a Streptomyces lividans gal operon galK gene or any functional derivative thereof and a foreign functional DNA sequence operatively linked to the galK gene, which comprises transforming an appropriate host microorganism or cell with such molecule.

22. A method of enabling a nongalactose utilizing host microorganism or cell to utilize galactose which comprises transforming such host with a recombinant DNA vector or molecule comprising Streptomyces gal operon, or any portion of the Streptomyces gal operon which is adequate to enable such transformed host to utilize galactose, or any functional derivative thereof.

Figure 1

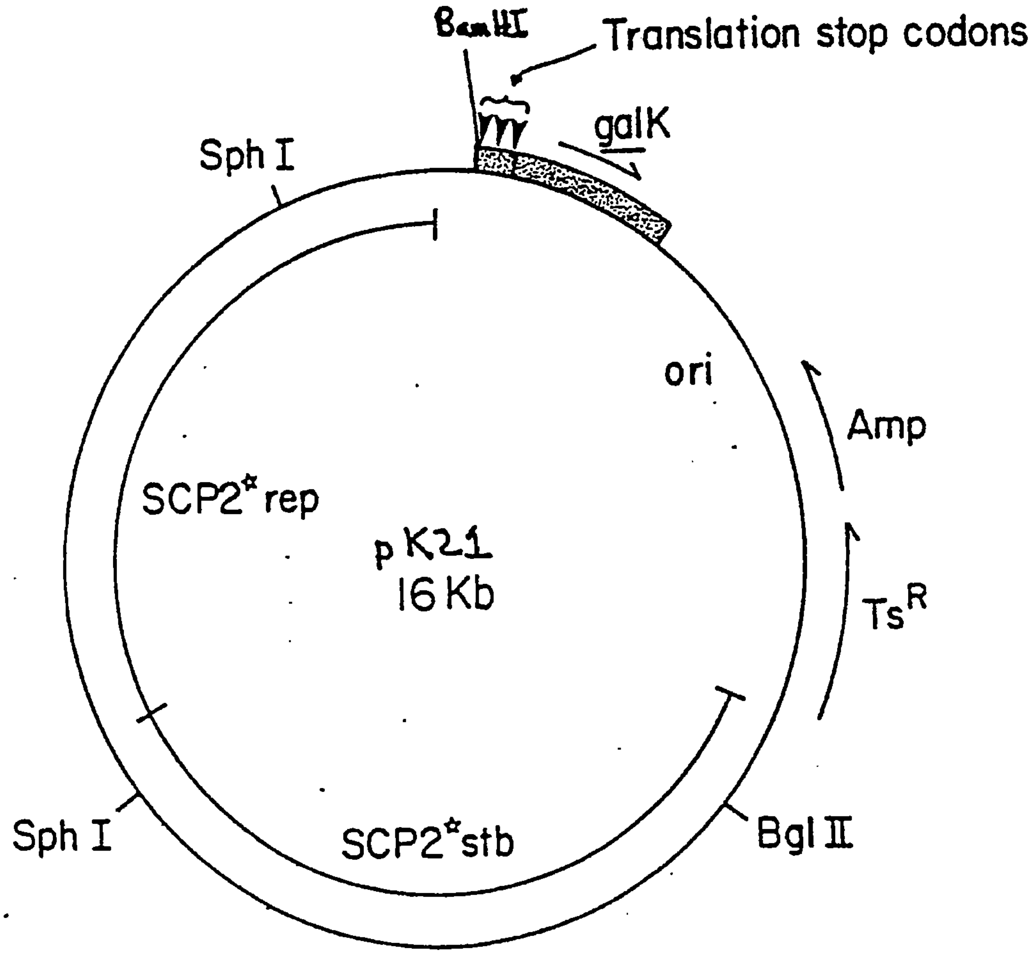
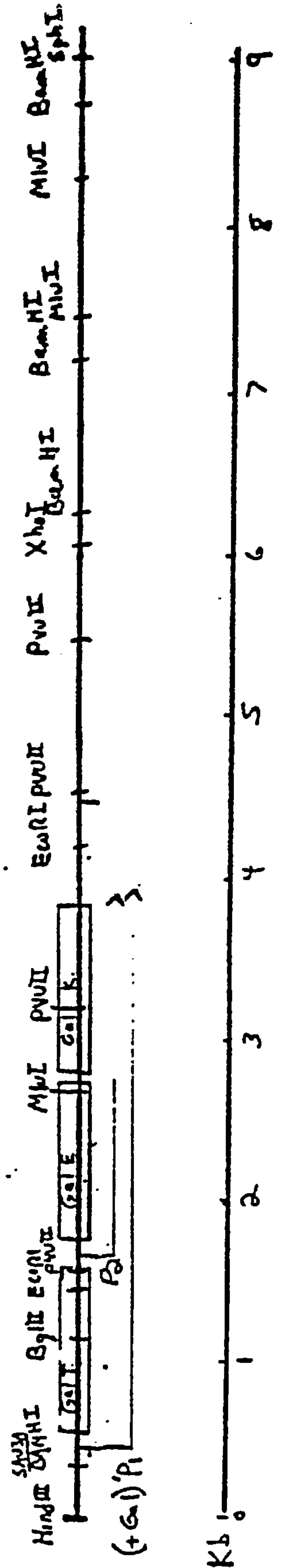

BamHI
Translation stop codons
galK
Sph I
ori
Amp
SCP2* rep
pK21
16 Kb
Ts^R
Sph I
SCP2* stb
Bgl II

Figure 2


HindIII
Sau3A
BamHI
BglII
EcoRI
PvuII
MluI
PvuII
EcoRI
PvuII
PvuII
XhoI
BamHI
BamHI
MluI
MluI
BamHI
SphI
Gal T
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P2
Kb
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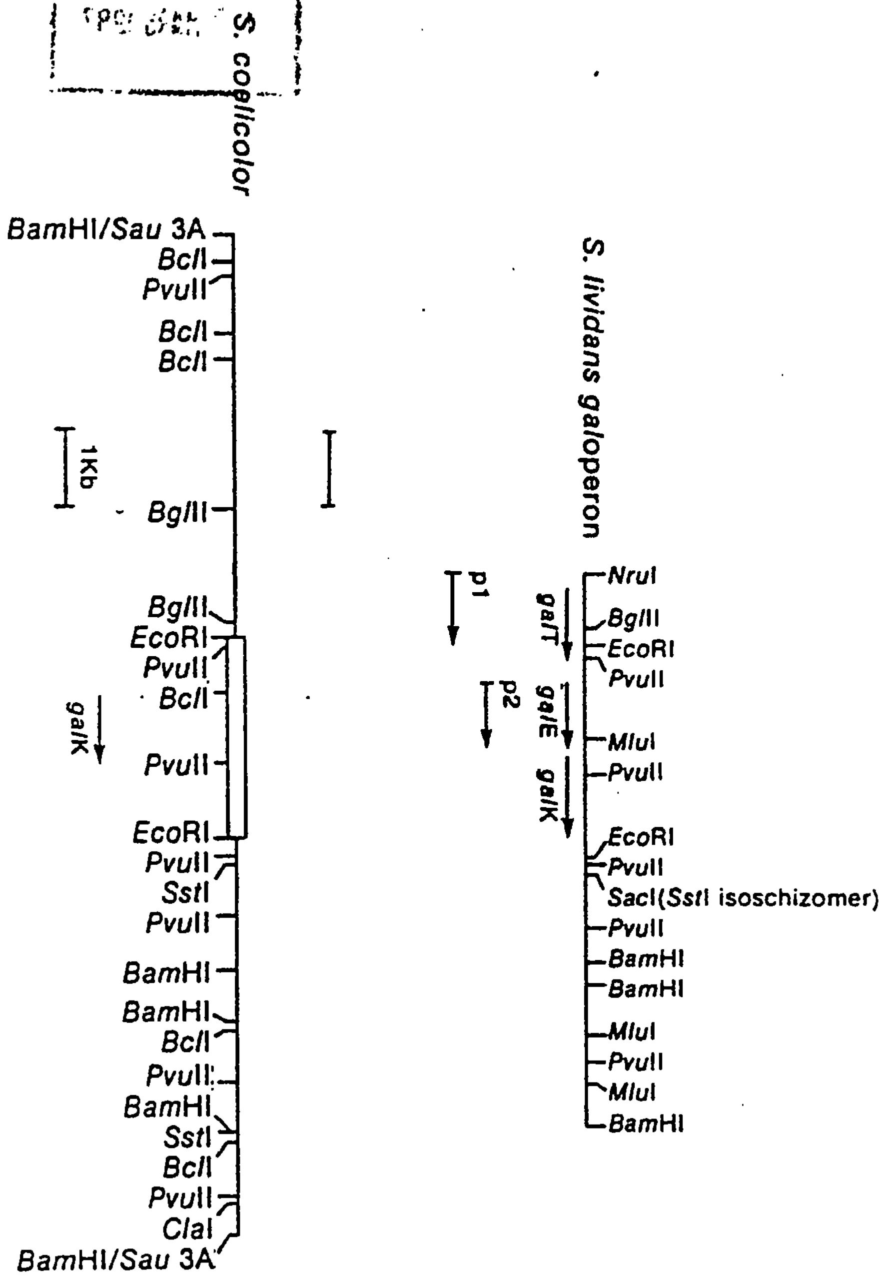
1
2
3
4
5
6
7
8
9

Figure 3


S. coelicolor
BamHI/Sau 3A
BclI
PvuII
BclI
BclI
1Kb
BglII
BglII
EcoRI
PvuII
BclI
galK
PvuII
EcoRI
PvuII
SstI
PvuII
BamHI
BamHI
BclI
PvuII
BamHI
SstI
BclI
PvuII
ClaI
BamHI/Sau 3A
S. lividans galoperon
p1
p2
galT
galE
galK
NruI
BglII
EcoRI
PvuII
MluI
PvuII
EcoRI
PvuII
SacI(SstI isoschizomer)
PvuII
BamHI
BamHI
MluI
PvuII
MluI
BamHI